

# **The efficacy of biological control agents of gorse, *Ulex europaeus* L., in Tasmania**

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Doctor of Philosophy

School of Agricultural Science and Tasmanian Institute of Agricultural Research

CRC for Australian Weed Management

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## **DECLARATION**

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This thesis contains no material that has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information, which has been duly acknowledged within the thesis. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where reference is made in the text.

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## Abstract

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The primary aim of this study was to assess the individual impact of the gorse seed weevil (*Exapion ulicis*), gorse spider mite (*Tetranychus lintearius*) and gorse thrips (*Sericothrips staphylinus*) on the growth and development of gorse. Factors such as natural enemy attack and herbicide use may affect biological control agent populations and their resulting impact on the target weed. For this reason, the study was extended to assess the potential impact of natural enemies on the gorse spider mite and the gorse thrips and assess the toxicity of herbicides and adjuvants commonly used for gorse control on the gorse thrips.

The gorse spider mite caused a reduction in dry matter production of approximately 36% in a field experiment that compared mite-attacked plants to sprayed controls over two and a half years. A threat to the effectiveness of this agent is predation by the Chilean predatory mite, *Phytoseiulus persimilis*. An experiment showed this species will develop at a similar rate on a diet of gorse spider mite as it would on the two-spotted mite, therefore confirming the ability of this species to have a negative impact on gorse spider mite populations.

In a glasshouse experiment, gorse thrips, ryegrass competition and simulated grazing individually reduced the growth of gorse seedlings. When treatments were combined, survival of gorse seedlings was also reduced. These results suggest that this species has the potential to be a useful biological control agent of gorse. However, as population build up in the field has been slow, further studies on the biology and ecology of this species are required. A field study was conducted to identify potential natural enemies of the gorse thrips within the arthropod fauna inhabiting gorse. A

range of species, including generalist predators, were identified. Of these, the Phlaeothripid *Haplothrips victoriensis* and mites in the family Phytoseiidae were the most abundant predatory arthropods present on gorse throughout the study and are also reported to be natural enemies of other members of the family Thripidae. Further studies are required to test the predatory efficacy of these arthropods on gorse thrips.

The gorse seed weevil was found to reduce seed production of gorse at two sites in Tasmania. Due to pod production being continual at Lymington and only over spring and summer at Stonehenge, the percentage of mature seed damaged in black pods for the whole 20 months of sampling was 2.7 times higher at Stonehenge (45.5%) than at Lymington (16.7%). On an annual basis, damage to mature seed at Stonehenge was 34.4% (2001/2002) and 55.4% (2002/2003) and at Lymington was 18.1% (2001/2002) and 12.4% (2002/2003). Based on the results of this study and population models produced elsewhere, it was concluded that additional seed feeding biological control agents would be required to reduce seed production of gorse to below replacement levels.

A bioassay was conducted to determine the toxicity of herbicides and adjuvants commonly used to control gorse on adult and juvenile gorse thrips. The herbicide triclopyr/picloram was found to be consistently the most toxic chemical to both juvenile and adult gorse thrips. Some toxicity was measured for the herbicide glyphosate, the adjuvant modified polydimethylsiloxane and the adjuvant soyal phospholipids/propionic acid. Metsulfuron methyl was not significantly toxic to either adult or juvenile gorse thrips. Both adjuvants seemed to increase the toxicity of all the herbicides on both juvenile and adult gorse thrips. The integration of chemical and biological control using the gorse thrips is discussed.

Although each individual agent did have a measurable impact on certain aspects of gorse performance, each had its limitations. Predation of the gorse spider mite by the specialist predator, *Phytoseiulus persimilis*, is likely to limit its impact on gorse. The gorse thrips did have an impact on gorse in a glasshouse environment but populations have been slow to establish in the field and no evidence of an impact of this species has been recorded five years after release. The gorse seed weevil does have an impact on the production of gorse seed, however, the levels recorded in this study were not enough to have an impact on gorse populations. The combined impact of these agents on gorse populations was beyond the scope of this study, however, due to limitations of each agent, it seems apparent that additional agents will be required if biological control is to be considered an important long-term component of an integrated management strategy for gorse.

## Acknowledgements

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## **Chapter 1. General Introduction**

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### **1.1 Integrated Weed Management**

Integrated weed management (IWM) is a decision making process with the aim of selecting and applying multiple, compatible control techniques in a sustainable manner to reduce weed populations below economically damaging levels. This approach uses multiple control methods such as biological, cultural, physical and chemical methods. Therefore, IWM requires a multi-disciplinary approach in order to develop a management system that is practical and cost effective in achieving a desired level of weed control. IWM is a form of ecosystem management and knowledge of the ecology of the weed and the invaded system are of fundamental importance as this will allow identification of weaknesses within the weed's lifecycle and appropriate control strategies can then be devised (Paynter and Flanagan, 2004).

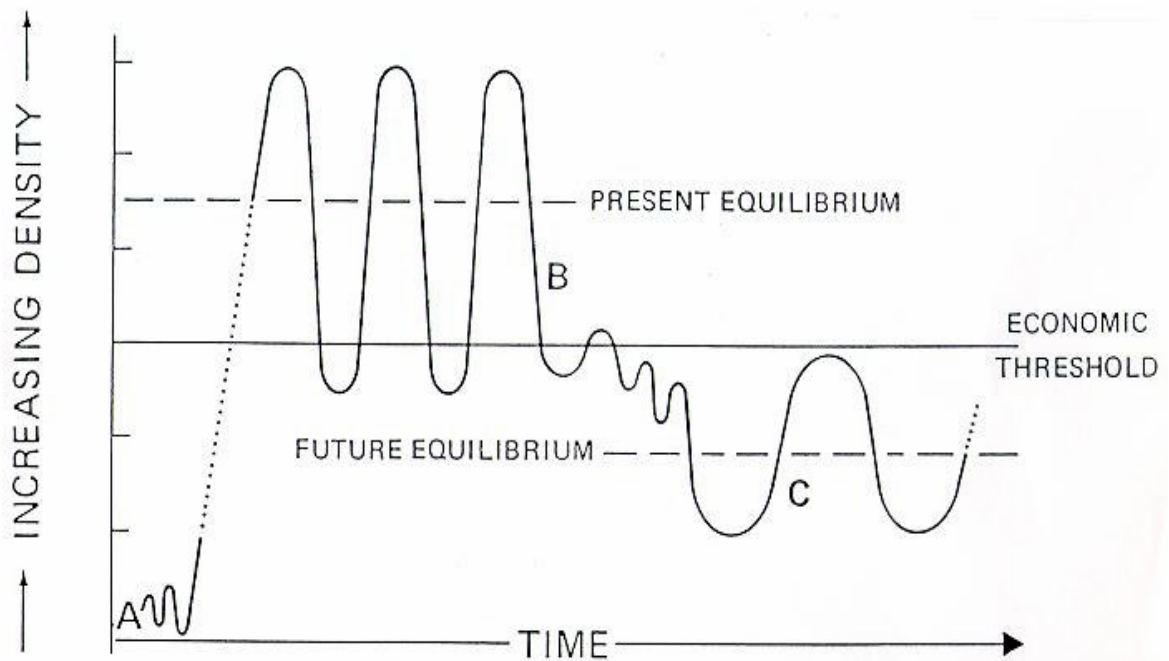
Monitoring of weed populations and accurate record keeping is also very important. This can assist in determining the timing of particular management strategies within an IWM strategy. Just as importantly, monitoring can allow a comparison of predicted versus actual outcomes of a particular strategy or combination of strategies and therefore allows fine-tuning of the IWM strategy.

## 1.2 Biological Control

When applied to invasive plant species, the 'enemy release hypothesis' predicts that plants will increase in abundance in an exotic region as they are not regulated by specialist natural enemies. According to this hypothesis, the introduction of specialist natural enemies as biological control agents should therefore cause a decrease in the abundance of the plant in the exotic region (Keane and Crawley, 2002).

Eilenberg *et al.*, (2001) defines four strategies of biological control: classical, conservation, inoculation and inundation. Classical biological weed control involves the deliberate introduction of host-specific exotic natural enemies (eg. phytophagous arthropods or fungal pathogens) with the expectation that an agent, or guild of agents, will become permanently established. Classical biological control is the predominant method in weed biological control. Other biological control methods are practised less often (McFadyen, 1998). The aim of classical weed biological control is to reduce a weed population to less damaging levels (a simplified conceptual diagram is illustrated in Fig. 1.1).

Biological control agents are usually sourced from the target weed in the country of origin where both the agent and weed have co-evolved. Biological control relies on a damaging interaction between the weed and the agent, and as this can be influenced significantly by a range of factors (discussed in section 1.3), levels of control can vary between locations, seasons and years.



**Figure 1.1.** Theoretical representation of a weed population, following initial weed introduction (A) the weed population will reach an equilibrium level (B), after the introduction of a biological control agent the weed population will reach a new equilibrium (C), if the biological control agent is successful, the new equilibrium (C) will be below the economic threshold (after Smith and van den Bosch, 1967).

### **1.3 Evaluating the impact of biological control agents**

A successful weed biological control agent should ultimately have a significant impact on the population dynamics of its host. However, evaluating such an impact on weed density is often constrained by time and resources. Therefore, the success of biological control should only be claimed after it has been shown that the agent(s) have a measurable impact on aspects of the target weed's performance. Post-release assessments should be conducted to quantify this impact and assess the competitive ability of the weed both with and without biological control agent(s). Post-release impact studies are rarely conducted (Blossey and Skinner, 2000), hence such evaluation will also help improve the efficiency and effectiveness of subsequent weed biological control programs by identifying common factors affecting agent impact. Even if the impact of an agent is determined to be damaging in evaluation studies, further studies are often required to determine if this impact translates into a widespread long-term impact on the weed population.

Evaluation of the impact of biological control is important in determining economic benefits and justifying continued funding support, however, this should not be the sole reason for evaluating the impact of an agent. An evaluation program will also provide a better understanding of the biology of the weed and agent and their interactions, and may also help determine the reasons for success or failure of a particular agent (Sheppard *et al.* 2003). This may help improve the agent selection process and our understanding of the theoretical processes of biological control. This knowledge may help to improve the integration of biological control with other control methods in an IWM program.

Ecological models can aid impact evaluation at both local and regional scales by explaining what is observed and providing a prediction of future population trends. These ecological models can then provide the basis of economic models that demonstrate the cost/benefits of weed biological control programs (Kriticos, 2003; Sheppard *et al.* 2003).

The development of methods to quantify the impact of a biological control agent on a target weed is still in its infancy. Three phases in evaluating a biological control program are recommended by Farrell and Lonsdale (1997): (1) Baseline studies on the population density, growth characteristics, biology and ecology of the weed are conducted before release of a biological control agent so that changes over time can be quantified. (2) Monitoring establishment and spread of an agent following release should be conducted to determine if establishment has been successful and to measure the rate of spread. (3) Evaluation of impact should be conducted once establishment has been confirmed in areas beyond the general release area.

Methods of assessing the impact of biological control agents can vary depending on the biology of both the weed and agent. In general, these assessments are often based on a comparison of parameters such as growth, seed production and survival of the weed with the agent(s) compared to a control which has the agent(s) excluded from the weed. Examples of published weed biological control agent impact studies, including three studies on gorse, are provided in Table 1.1

**Table 1.1.** Examples of studies that have assessed the impact of arthropod biological control agent(s) on weeds.

Target weed <sup>1</sup>	Biological control agent(s) <sup>2</sup>	Type of study <sup>3</sup>	Assessment method <sup>4</sup>	Parameters assessed <sup>5</sup>	Reference
<i>Acacia nilotica</i> (WS)	<i>Bruchidus sahlbergi</i> (SF), <i>Caryedon serratus</i> (SF)	F	NE	Sd	Radford <i>et al.</i> (2001)
<i>Carduus nutans</i> (B)	<i>Trichosirocalus horridus</i> (RC)	F	IE	VG, Sd, P	Woodburn (1997)
<i>Cirsium arvense</i> (P)	<i>Cassida rubiginosa</i> (RC)	F	BCT, PC	VG, RG, P	Bacher and Schwab (2000)
<i>Echium plantagineum</i> (A)	<i>Mogulones larvatus</i> (RC)	F	IE, PC	VG, Fl, Sd, P	Sheppard <i>et al.</i> (2001)
<i>Hieracium pilosella</i> (HP)	<i>Aulacidea subterminalis</i> (GF)	NF	CE, PC	VG, RG	Kloppel <i>et al.</i> (2003)
<i>Lythrum salicaria</i> (PW)	<i>Gallerucella calmariensis</i> (FF)	F	NE, PC	VG, Fl, P	Landis <i>et al.</i> (2003)
<i>Lythrum salicaria</i> (PW)	<i>Gallerucella calmariensis</i> (FF), <i>G. pusilla</i> (FF)	F	NE, PC	Fl, Sd	Katovich <i>et al.</i> (2001)
<i>Mimosa pigra</i> (WS)	<i>Neurostrotta gunniella</i> (SB), <i>Phloeospora mimosae-pigrae</i> (FP)	NF	CE	VG	Paynter and Hennecke (2001)
<i>Mimosa pigra</i> (WS)	<i>Neurostrotta gunniella</i> (SB)	F	IE, PC	VG, Sd, P	Lonsdale and Farrell (1998)
<i>Onopordum</i> thistles (B/HP)	<i>Trichosirocalus briesei</i> (RC)	F	CE	VG, Fl, Sd, P	Briese <i>et al.</i> (2002)
<i>Onopordum</i> thistles (B/HP)	<i>Larinus latus</i> (SF)	F	NE	Sd	Briese (2000)
<i>Onopordum</i> thistles (B/HP)	<i>Lixus cardui</i> (SB)	F	CE	VG, Fl, Sd	Briese (1996)
<i>Parthenium hysterophorus</i> (A)	<i>Epiblema strenuana</i> (GF)	F	CE, PC	VG, Fl, Sd	Dhileepan and McFadyen (2001)
<i>Parthenium hysterophorus</i> (A)	<i>Epiblema strenuana</i> (GF)	NF	BCT, PC	VG, Sd	Navie <i>et al.</i> (1998)
<i>Parthenium hysterophorus</i> (A)	<i>Zygogramma bicolorata</i> (FF), <i>Epiblema strenuana</i> (GF)	F	IE	VG, Fl, Sd	Dhileepan (2001)
<i>Parthenium hysterophorus</i> (A)	<i>Zygogramma bicolorata</i> (FF)	F, NF	CE	VG, Fl, Sd, RG	Dhileepan <i>et al.</i> (2000)

**Table 1.1 (continued).** Examples of studies that have assessed the impact of arthropod biological control agent(s) on weeds.

Target weed <sup>1</sup>	Biological control agent(s) <sup>2</sup>	Type of study <sup>3</sup>	Assessment method <sup>4</sup>	Parameters assessed <sup>5</sup>	Reference
<i>Sarothamnus scoparius</i> (WS)	Numerous arthropods	F	IE	VG, Sd, P	Waloff and Richards (1977)
<i>Senecio jacobaea</i> (B/HP)	<i>Longitarsus flavicornis</i> (RC)	F	NE	P	Ireson <i>et al.</i> (1991)
<i>Senecio jacobaea</i> (B/HP)	<i>Longitarsus jacobaeae</i> (RC)	F	NE, PC	VG, P	Windig (1993)
<i>Ulex europaeus</i> (WS)	<i>Exapion ulicis</i> (SF)	F	IE	Sd	Norambuena and Piper (2000)
<i>Ulex europaeus</i> (WS)	<i>Sericothrips staphylinus</i> (FF)	NF	IE, PC	VG, RG, P	Davies <i>et al.</i> (2005)
<i>Ulex europaeus</i> (WS)	<i>Sericothrips staphylinus</i> (FF), <i>Tetranychus lintearius</i> (FF), <i>Dictyonota strichnocera</i> (FF)	NF	CE	VG	Fowler and Griffin (1995)

**Codes**

1: A = annual; B = biennial; HP = herbaceous perennial; WS = woody shrub; PW = perennial wetland plant

2: FF = foliage feeder; SF = seed feeder; GF = gall former; SB = stem borer; FP = fungal pathogen; RC = root or crown feeder

3: F = field study; NF = non-field study (eg. glasshouse or shadehouse)

4: BCT = biocontrol treatment compared to control but exclusion not necessary; IE = insecticide exclusion; CE = cage exclusion; NE = non-exclusion study (eg. assessment based on surveys); PC = plant competition also assessed

5: VG = vegetative growth assessed; RG = root growth assessed; Fl = flowering assessed; Sd = seed production assessed; P = populations or weed mortality assessed



The level of impact exerted by a given biological control agent is often quite variable. Many factors can either negatively or positively influence the population dynamics of either the agent(s) or weed. These changes in populations may then influence the impact of the agents on the target weed. The following factors, not exclusive or in order of importance, may influence the impact of biological control: environmental conditions (eg. van Klinken, 2004), genetic variability/compatibility of the target weed and biological control agent (eg. Thomas and Ellison, 2000), biotic factors (eg. predation, parasitism and competition) (Pratt, *et al.* 2003; Ireson, *et al.*, 2002; Goeden and Louda, 1976) and weed or crop management strategies (Rees and Hill, 2001; Sheppard, 1996).

## **1.4 Gorse**

### *Distribution and weed status*

Gorse, *Ulex europaeus* L. (Fabaceae) (Figs. 1.2 and 1.3), is native to Great Britain and central and western Europe, where it occurs in native heathland, forests and neglected or disturbed farmland (Parsons and Cuthbertson, 2001; Zwölfer 1962). Gorse has been introduced to many countries world-wide and has become an important weed in Australia, New Zealand, Chile, India, and western USA and Hawaii (Richardson and Hill, 1998; Parsons and Cuthbertson, 2001). Gorse was originally introduced to Australia as an ornamental hedging plant and was listed in a Tasmanian nursery catalogue in 1845. It was thought to be present in Tasmania in the early 1800's and was reported as naturalised in 1889 (Parsons and Cuthbertson, 2001). Gorse occurs in Tasmania, Victoria, South Australia, Western Australia and New South Wales (Fig. 1.4 a) and is a Weed of National Significance (Thorp, 1999). Gorse has a serious

impact on agricultural land and environmentally significant regions in South Eastern Australia, predominantly Victoria (Fig. 1.4 b) and Tasmania (Fig. 1.4 c).

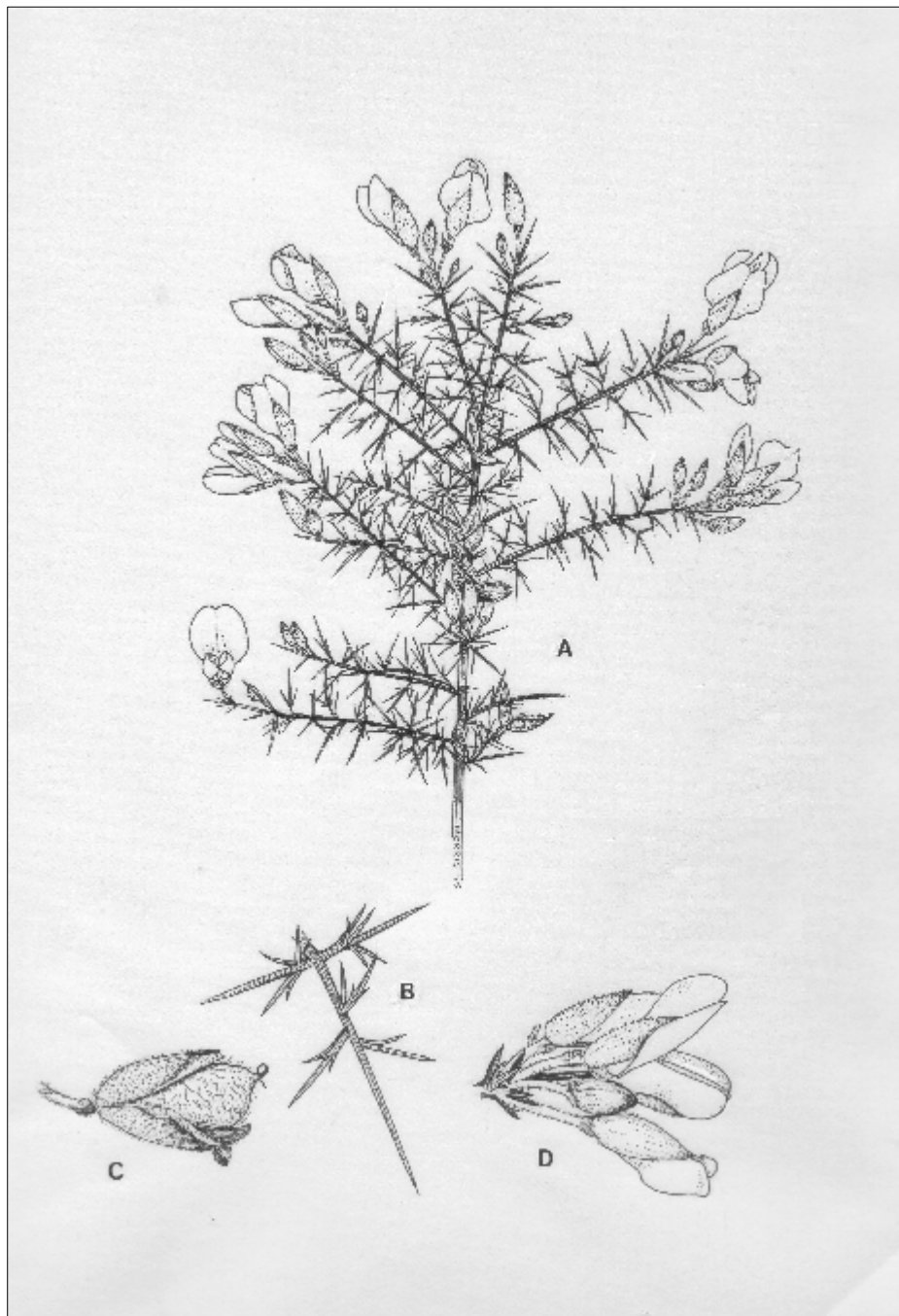
In Tasmania gorse grows in areas from sea level to 800 m in altitude with an annual rainfall of 500-1500 mm (Fig. 1.4c). The heaviest infestations, covering approximately 30 000 ha, occur in the central and northern midlands on pastures grazed mainly by sheep (Ireson *et al.* 1999). Isolated heavy infestations also occur on the West Coast, in the far north west in the Circular Head district, on the East Coast and South East region, the far north east around Gladstone and in the George Town area, gorse is also present on King Island.

In Victoria, Lane *et al.* (1980) listed gorse as the sixteenth most widespread weed. Their surveys showed that it occupied an estimated total area of 948 000 hectares (with scattered infestations on 805 000 ha. and medium to dense infestations on 143 000 ha.). It is common along roadsides and on disturbed land in the central highlands region, south west Victoria and parts of Gippsland. It also extends into the eastern, south eastern and south west fringes of the grain belt (Fig. 1.4 b).

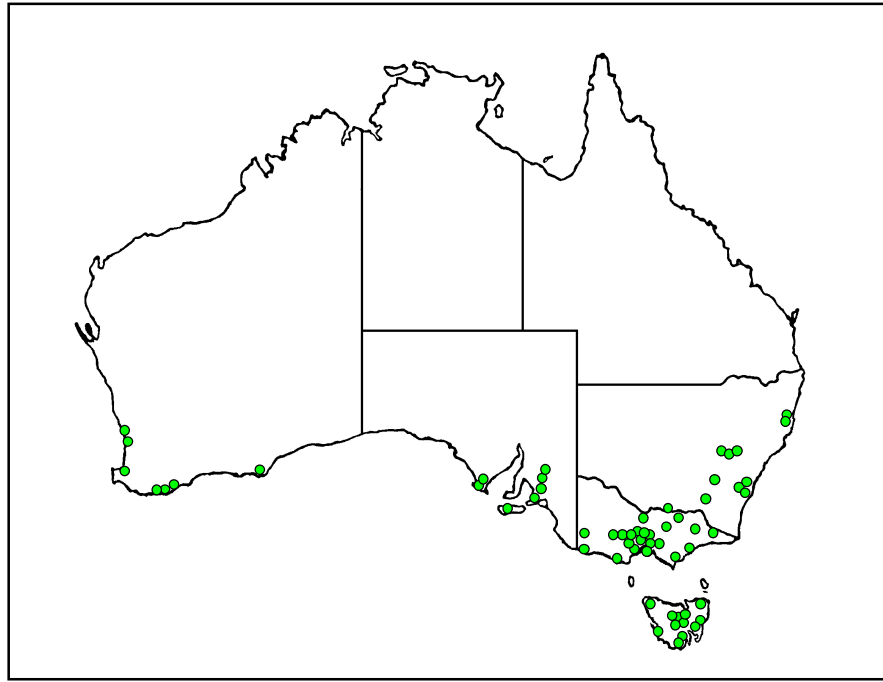
Gorse is not as big a problem in other mainland states (Fig 1.4 a). In South Australia gorse has a scattered distribution in the higher rainfall areas of the state, particularly in the Mt. Lofty ranges, and has also been recorded on Kangaroo Island. Gorse is uncommon in Western Australia, where it is reported from a total of 175 locations covering an estimated area of 185 ha, the main areas affected are in the southwest of the state around Albany. In NSW it has a very limited distribution but is locally common on the north and central coasts, central tablelands and central and southwest slopes (Parsons and Cuthbertson, 2001).



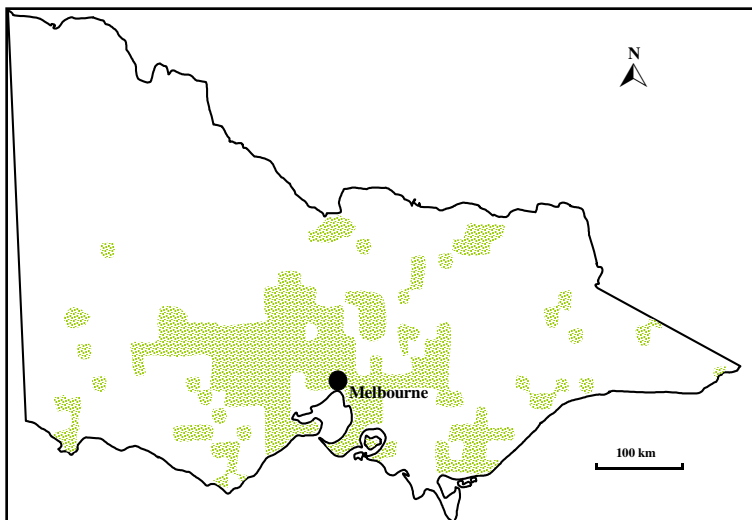
**Figure 1.2.** Gorse (*Ulex europaeus*) infestation, Lymington, Tasmania (Courtesy Tasmanian Institute of Agricultural Research).



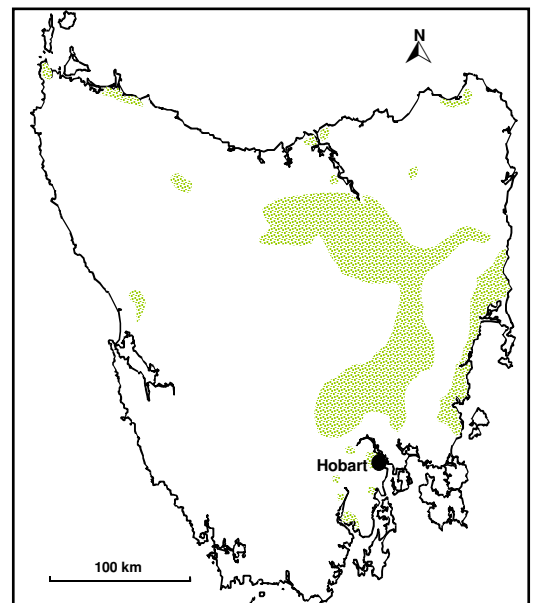
**Figure 1.3.** Gorse (*Ulex europaeus*) (a) Flowering branch (b) Spine (c) Pod (d) Flowers (Courtesy DPIWE, Tasmania).



(a)



(b)



(c)

**Figure 1.4.** Distribution of gorse (*Ulex europaeus*) in (a) Australia (b) Victoria (c) Tasmania (Courtesy Tasmanian Institute of Agricultural Research).

In the major problem areas of Tasmania and Victoria, gorse is considered a serious weed because it invades pastoral land thereby reducing pasture and animal productivity and also providing habitat and shelter for vertebrate pests (Richardson and Hill, 1998). In forestry plantations it reduces tree growth and survival and increases the risk of fire. In addition, gorse invades native bushland thereby reducing access and conservation values, increasing fire hazards and threatening the survival of rare and endangered plants and plant communities (Richardson and Hill, 1998).

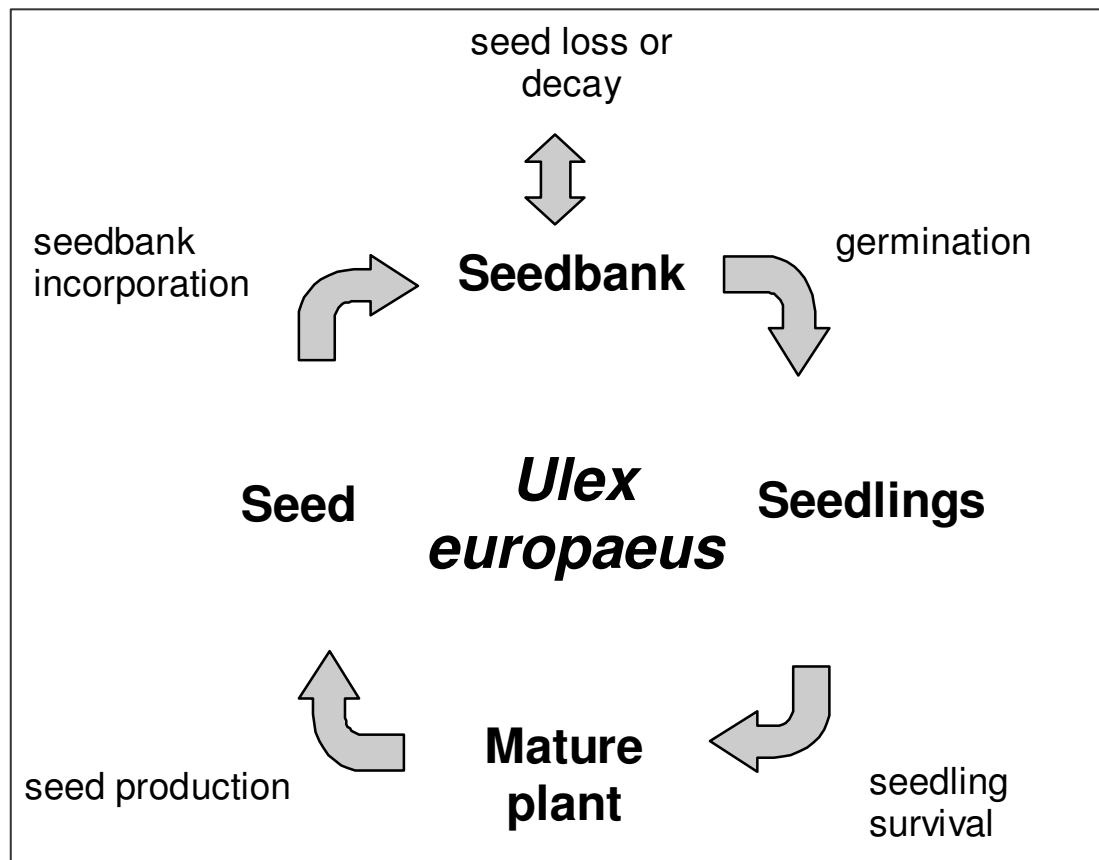
### *Biology*

Gorse is a spiny, leguminous, woody shrub which can live up to 30 years of age and grow to more than 3m in height and diameter (Parsons and Cuthbertson, 2001). Gorse flowers (Fig. 1.3 a and d) are primarily produced in spring but the production of flowers outside this period, particularly in autumn and winter, is common. Seed is produced in small pods (Fig. 1.3 c) following flowering mainly in late spring and summer. Large numbers of seed are normally produced and can remain viable in soil in excess of 25 years (Moss, 1959). Seed dispersal is mainly via dehiscence from pods but also via animals, man and water. The distance of ejection from dehiscence is limited, it is thought that 5 metres is the maximum distance and that most seed are dispersed less than 2.5 metres from bushes (Richardson and Hill, 1998). In addition, Norambuena and Piper (2000) found that gorse seed density declined significantly with increasing distance from the parent plant. As seed dispersal is limited, most of the seed falls within the thicket where a large, long-lived seed bank rapidly develops in the litter and soil. Seeds are often stimulated into germination by fire or mechanical disturbance, therefore following such a disturbance, plants in a gorse thicket are often of a similar age. As gorse is such an abundant producer of long-lived seed, seedling

establishment and survival on disturbed ground is an important component of gorse population dynamics (Rees and Hill, 2001). The major processes in the gorse lifecycle are displayed in Fig. 1.5.

In a natural plant community, gorse is a pioneering species that readily invades disturbed ground (Rees and Hill, 2001) and often dominates early on in the plant community succession process. Following a disturbance event, such as fire or mechanical clearing, gorse rapidly forms thickets from a long-lived seed bank and/or living stumps. These thickets form dense, impenetrable monocultures (Fig. 1.2) that compete with pasture and other desirable plant species. Gorse can grow at a rapid rate in many soil types, in poor soils gorse is particularly competitive presumably partly due to its nitrogen fixing properties.

As gorse has such a long-lived seed bank and is such a competitive pioneering species, it can be particularly difficult to control. Chemical, cultural and biological control options are discussed in the remaining sections.



**Figure 1.5.** Schematic representation of the lifecycle of gorse (*Ulex europaeus*).



## **1.5 Chemical Control of Gorse**

Results from trial work in Tasmania indicate that the most effective herbicide for gorse control is a mixture of triclopyr and picloram (eg. Grazon DS<sup>®</sup>) (Anonymous, 1997). Where thorough coverage of the bush can be achieved, one application will give complete control with no regrowth. However, it is recommended that treated bushes be checked 12 months after application and the re-growth treated. Because of the sensitivity of clover, horticultural crops and trees to the picloran component of Grazon, the chemical is not recommended for use in orcharding, or horticultural cropping areas or where desirable tree species are present. Triclopyr alone or alternate herbicides such as metsulfuron-methyl, amitrole or glyphosate, although less effective than Grazon, are recommended when the use of Grazon is inappropriate (Anonymous, 1997). Grazon can be applied throughout the year.

All herbicides used for control of gorse are severely damaging to pasture legumes and desirable trees and shrubs. Damage to eucalypts, wattles and other non-target species is common where gorse is controlled by foliar application of herbicides in bushland (Anonymous, 1997). Picloram, one of the component herbicides of the most commonly used product (Grazon DS), can persist in the ground for up to two years and prevent re-establishment of pasture legumes in treated areas (Anonymous, 1997).

## **1.6 Cultural Control of Gorse**

### *Burning*

Burning alone will not adequately control gorse (Anonymous, 1997). By itself, burning is only a stop-gap measure as regrowth of established bushes and seedling

establishment are generally rapid after burning. Burning reduces the amount of foliage drastically and produces green shoots which are presumably far more attractive to goat or sheep browsing than mature shoots. Burning is also useful if done several months after spraying when, under the best conditions, it reduces even the heaviest of woody stems to ashes.

### *Mechanical clearing, cultivation and mulching*

Mechanical clearing followed by cultivation is an effective method for removing large infestations on land that is suitable for sowing down to pasture. Bulldozers with rippers, or medium or heavy tractors with dozer blades and rippers attached can be used. The object of mechanical clearing is to rip out as much of the root system as possible to reduce regrowth. This work should therefore be conducted when the ground is soft to maximise root removal. The major level of soil disturbance associated with mechanical removal of gorse followed by cultivation leaves treated areas susceptible to soil erosion and reinvasion by gorse or other weeds.

Mulching using a heavy-duty rotary hoe pulverises all the above-ground plant material and incorporates it into a mulch. The advantage of this method is that major soil disturbance is avoided (Swan and Faithful, 2004). This is an effective method of controlling mature gorse stands but is restricted to stone free ground.

Follow up controls need to be implemented after mechanical clearing, cultivation and mulching to prevent rapid re-infestation from roots, stumps and seed.

### *Grazing*

Although grazing by livestock is considered a biological control method by some authors it is considered in this section as a management or cultural practise rather than

biological control in the traditional sense (see section 1.7 for a definition of biological control). Grazing by sheep is an effective method for controlling gorse seedlings (Anonymous, 1997). After a dense gorse infestation has been removed and the area sown to pasture it can be grazed heavily by sheep during the spring and summer to prevent the establishment of gorse seedlings.

Sheep will browse established gorse bushes during spring or when alternative feed is in short supply. However, they prefer to eat pasture species so that significant control cannot be achieved by sheep grazing unless large numbers are confined to gorse patches for most of the year (Anonymous, 1997).

Harradine and Jones (1985) have shown that Angora goats are ideal for gorse control. Goats prefer to browse young gorse shoots rather than graze actively growing pasture. They remove flowers and defoliate bushes, browsing them back to stumps when the stocking rate is high enough. However, well-established gorse bushes are not readily killed by browsing and are capable of recovery after several years of browsing if the goats are removed from the area.

## **1.7 Biological Control of Gorse**

Biological control programs are being implemented in several countries around the world in the exotic range of gorse. Hawaii and west coast states of the USA (Markin *et al.*, 1996; Richardson and Hill, 1998), New Zealand (Richardson and Hill, 1998) and Chile (Norambuena *et al.*, 2000) all have established biological control programs for gorse.

In Australia, gorse was approved as a target for biological control in July 1995, following nomination by the then Department of Primary Industry and Fisheries, Tasmania (Ireson *et al.*, 1999). If successful, biological control may offer significant economic benefits. If the introduction of an agent or guild of agents has a significant impact on the population dynamics of gorse, then significant saving may be had due to increased productivity and reduced costs of other control methods. Additional benefits may also arise in environmentally significant areas due to an increase in native flora if a reduction in the competitiveness of gorse can be achieved. Three biological control agents of gorse have so far been introduced into Australia.

#### *Gorse seed weevil*

The first agent to be introduced into Australia as a biological control agent for gorse was the gorse seed weevil, *Exapion ulicis* (Forster) (Coleoptera: Brentidae) (Fig. 1.6). This species was originally from Europe but was introduced in 1939 from a population that had previously established in New Zealand (Evans, 1943). Adult female weevils (Fig. 1.6 a) chew a hole in gorse pods and oviposit into the pods. The oviposition hole heals over, eggs hatch and larvae (Fig. 1.6 b) develop within the pods feeding on the developing seed within thereby reducing gorse seed production. Pupation takes place inside the pods (Fig 1.6 c) and adult gorse seed weevils emerge when the seed ripens and pods dehisce.



(a)



(b)



(c)

**Figure 1.6.** Gorse seed weevil (*Exapion ulicis*) (a) Adult on gorse flower (Courtesy Tasmanian Institute of Agricultural Research) (scale 1:10) (b) Larvae in damaged gorse pod (left) with an undamaged pod (right) (Courtesy Tasmanian Institute of Agricultural Research) (scale 1:3) (c) Pupae in damaged gorse pod (scale 1:10).

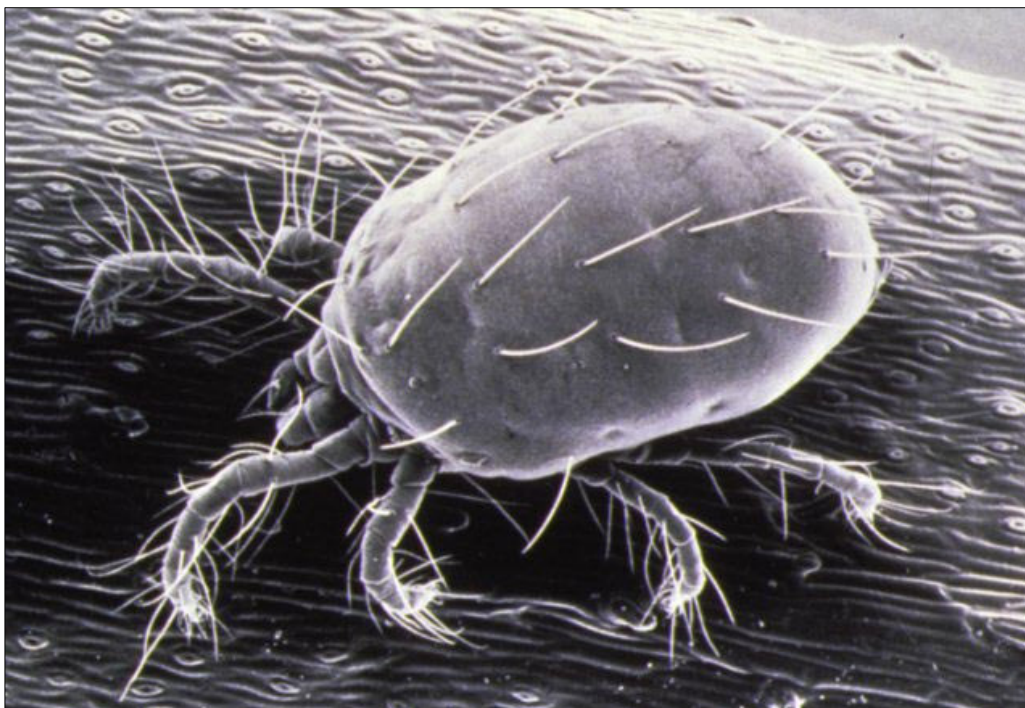
### *Gorse spider mite*

The gorse spider mite, *Tetranychus lintearius* Dufour (Acari: Tetranychidae) (Fig. 1.7 a) was released in Tasmania and Victoria in 1998 following host specificity testing on over 130 plant species (Hill and O'Donnell, 1991 a; Ireson *et al.* 2003). This species feeds preferentially on mature gorse foliage using its piercing and sucking mouthparts to puncture cells and remove cellular contents. This process changes physiological processes such as decreasing the photosynthetic rate and altering the transpiration rate (Tomczyk and Kropczynska, 1985), which may reduce plant growth. Feeding by the gorse spider mite in high numbers results in a distinctive widespread 'bleaching' damage to gorse (Fig. 1.7 b).

### *Gorse thrips*

The most recent introduction of a biological control agent for gorse in Australia is the gorse thrips, *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae) (Fig. 1.8). This species is of European origin but was introduced to Australia in January 2001 from a population previously established in New Zealand. *S. staphylinus* of English origin (via New Zealand) was first released in Tasmania and Victoria in early 2001. Since then this species has been released at 237 Tasmanian and 73 Victorian sites (Ireson *et al.*, 2006). Fowler and Griffin (1995) suggest that gorse thrips can reduce the growth of gorse seedlings and Hill *et al.* (2001) observed heavy damage to potted gorse plants under laboratory conditions with visible damage present at some release sites in the field. This damage is caused by larval and adult gorse thrips feeding on mesophyll tissue. High numbers of gorse thrips produce distinct pale, stippled damage symptoms and abundant small frass droplets.





**(a)**



**(b)**

**Figure 1.7.** Gorse spider mite (*Tetranychus lintearius*) **(a)** Adult (Scanning Electron Micrograph, courtesy of Landcare Research, New Zealand) (scale 1:100) **(b)** Webbing and damage on gorse.



**Figure 1.8.** Adult gorse thrips (*Sericothrips staphylinus*) (Courtesy of Tasmanian Institute of Agricultural Research) (scale 1:70).



## 1.8 Integrated Management of Gorse

As stated previously (section 1.1), integrated weed management is the process of selecting and applying multiple, compatible control techniques to reduce weed populations below economically damaging levels. Therefore an integrated weed management strategy for gorse should combine the chemical, cultural and biological control methods previously outlined in a complementary manner to achieve effective control. A standardised monitoring program should also be conducted to record changes in weed populations. Management techniques for gorse are reviewed in Parsons and Cuthbertson (2001), Richardson and Hill (1998) and King, *et al.* (1996). The combination of techniques used in an integrated management strategy will vary according to land use and accessibility.

A strategic plan for gorse management in Australia (ARMCANZ and ANZECCFM, 2003) has a vision of managing gorse through collective action to minimise its social, economic and environmental impacts. The strategic plan has four components: best practise management of established infestations, prevention of spread, eradication of isolated infestations and management of 'at risk' areas.

Irrespective of the control methods employed, the prevention of reinfestation by gorse or of infestation by other weeds as a result of the removal of gorse cover is a matter of great importance. Before control or eradication is attempted there should be a clear idea of how the land is to be used and treated afterwards. For instance, the establishment of a vigorous, correctly fertilised permanent grass and clover sward will do much to suppress seedlings and will also allow heavier stocking rates. Grazing is an important factor in preventing recolonisation in cleared areas. Regrowth and any surviving young plants can be spot sprayed.

A combination of currently used methods ie. the use of chemicals, burning, cultivation and grazing can contain the problem on agricultural land and other mainly accessible areas. However, gorse is also a serious environmental weed in disturbed areas of a variety of vegetation types (Wells, 1991; Anonymous, 1997). The use of chemical and cultural control methods to contain its spread into areas of native vegetation is restricted because of the risk of damage to surrounding desirable species and limited accessibility.

## **1.9 Thesis aims**

The primary aim of this study was to assess the individual impacts of the gorse seed weevil, gorse spider mite and gorse thrips on the growth and development of gorse. Factors such as natural enemy attack and herbicide use may affect biological control agent populations and their resulting impact on the target weed. For this reason, the study was extended to assess the potential impact of natural enemies on the gorse spider mite and gorse thrips and assess the toxicity of herbicides and surfactants/adjuvants commonly used for gorse control on gorse thrips.

The contents and aims of the remaining chapters are summarised as follows:

*Chapter 2.* The impact of the gorse spider mite on the growth and development of gorse.

This field trial investigated the impact of the gorse spider mite on the growth and development of gorse over a period of 2.5 years in central Tasmania. A secondary aim of this investigation was to determine if the gorse spider mite had an interference

effect on another agent, the gorse seed weevil. The arrival of specialist tetranychid natural enemies was documented.

*Chapter 3. Development rates of the predatory phytoseiid mite, *Phytoseiulus persimilis*, on diets of pest and beneficial tetranychid mites.*

A controlled temperature development study was conducted to determine if two strains of the predatory mite, *Phytoseiulus persimilis*, would develop at similar rates when fed on diets of the gorse spider mite, *T. lintearius* and the two spotted mite, *T. urticae*. The development times of *P. persimilis* obtained in this and other studies were then compared to the development times of *T. urticae* and *T. lintearius* determined from other studies. The implication of these results for the effectiveness of *T. lintearius* as a biological control for gorse is discussed.

*Chapter 4. The phenology of the gorse seed weevil.*

A survey of gorse pods was conducted at two sites over two years to determine the period of gorse pod production and the levels of gorse seed weevil attack.

*Chapter 5. The impact of gorse thrips, ryegrass competition and simulated grazing on gorse seedling performance in a controlled environment.*

As the gorse thrips is a recent introduction into Australia and field populations are at low densities and not widely dispersed, a field study was not considered practical. Therefore, this study tested the impact of gorse thrips, ryegrass competition and simulated grazing on the growth and mortality of gorse seedlings in a glasshouse environment, as a first step towards the full assessment of their impacts in the field.

*Chapter 6.* Potential natural enemies of the gorse thrips within the arthropod fauna inhabiting gorse.

A field study was conducted with the aim of identifying potential natural enemies of the gorse thrips within the arthropod fauna inhabiting gorse in southern Tasmania.

*Chapter 7.* A preliminary assessment of the toxicity of commonly used herbicides and adjuvants used in gorse control on the gorse thrips.

A laboratory bioassay was conducted with the aim of determining the toxicity of commonly used herbicides and adjuvants for gorse control on adult and juvenile gorse thrips.

*Chapter 8.* General Discussion

The major findings of the thesis are summarised and the role of biological control within an integrated management plan for gorse is discussed.

### **1.10 Format of thesis chapters**

Each chapter in this thesis has been written to "stand alone", as in the format of a published journal article. As a result, introductory text is sometimes repeated within each chapter. To best fit the format of a thesis, acknowledgments and references have been pooled and figures and tables are numbered consecutively within each chapter.

## **Chapter 2. The impact of the gorse spider mite, *Tetranychus lintearius*, on the growth and development of gorse, *Ulex europaeus*.**

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### **Abstract**

A replicated field experiment was conducted to assess the impact of *Tetranychus lintearius* on the growth and reproductive capacity of gorse over two and a half years in Tasmania, Australia. The presence of *T. lintearius* caused a significant reduction in dry matter production of approximately 36%. This figure was calculated after control plants sustained unforeseen damage by *T. lintearius*. In the absence of *T. lintearius* damage to controls, it is predicted that a reduction in dry matter production caused by *T. lintearius* would have been approximately 44%. Two specialist predators of *Tetranychus* spp., *Phytoseiulus persimilis* and *Stethorus* sp., were both found at the site within the first year of the trial. *T. lintearius* was not found to negatively effect the damage caused by an established gorse biocontrol agent, the seed feeding weevil, *Exapion ulicis*. The use of *T. lintearius* as a biological agent for gorse in Australia, the interaction between agents and the impact of predators are discussed.

## 2.1 Introduction

Gorse, *Ulex europaeus* L. (Fabaceae), is a perennial, spiny shrub which can form dense, impenetrable thickets that compete strongly with pasture and other desirable plant species (Richardson and Hill, 1998). Gorse is a Weed of National Significance (Thorp, 1999) and has a serious impact on agricultural land and environmentally significant regions in South Eastern Australia. As part of an integrated management strategy, a guild of host-specific biological control agents are currently being introduced to gorse in Australia (Ireson *et al.*, 2004).

One of these agents, the gorse spider mite, *Tetranychus lintearius* Dufour (Acari: Tetranychidae), has been introduced to several countries where gorse is a serious weed including New Zealand (Richardson and Hill, 1998), Chile (Norambuena *et al.*, 2000), Australia (Ireson *et al.*, 2003), Hawaii and Pacific coast states of the USA (Markin *et al.*, 1996). *T. lintearius* is of European origin but was introduced to Australia in 1998 using populations previously established in New Zealand. It is now distributed throughout Tasmania, is widespread in Victoria and is established in southern New South Wales (Ireson *et al.*, 2006). *T. lintearius* is considered to be a promising biological control agent for gorse as high levels of damage have been observed in the native range of both species in Europe (Zwolfer, 1963), it is capable of producing several generations a year (Stone, 1986), is host specific to members of the genus *Ulex* (Hill and O'Donnell, 1991 a, Ireson *et al.*, 2003) and is reproductively isolated from pest *Tetranychus* species (Hill and O'Donnell, 1991 b).

Mites in the family Tetranychidae use piercing and sucking mouthparts to puncture cells and remove cellular contents. This process changes physiological processes such as decreasing the photosynthetic rate and altering the transpiration rate (Tomczyk and

Kropczynska, 1985), which can reduce plant growth and even alter flowering and seed production patterns. Feeding by *T. lintearius* in high numbers can result in a distinctive widespread 'bleaching' damage to gorse.

During surveys for potential biological control agents of gorse in Western Europe, Zwolfer (1963) observed large populations of *T. lintearius* on gorse and stated that heavy infestations of *T. lintearius* could result in the death of single branches or of the whole plant. However, this statement was based on visual assessments without supporting experimental data. In the only published experimental study of the impact of *T. lintearius* on gorse, Fowler and Griffin (1995) found that a treatment of *T. lintearius* significantly reduced shoot growth of gorse plants by approximately 50% over 34 days in the United Kingdom. In a New Zealand study (by P. McGregor, Landcare Research New Zealand Ltd., unpublished data), a *T. lintearius* treatment caused growth reductions of approximately 18% of whole plant weight after two successive years of attack. Richardson and Hill (1998) also cite the unpublished data from another New Zealand study (by T.R. Partridge) which showed that "heavy feeding by mites over consecutive seasons can kill individual shoots and cause severe reduction in the growth of plants in the third year". In another unpublished study conducted in Oregon, USA, Rice (2004) concludes that under certain conditions *T. lintearius* can reduce the growth of gorse over one season.

The biology of *T. lintearius* has been investigated by observing mites on enclosed gorse shoot tips (Stone, 1986). In that study, the total development times of female *T. lintearius* from egg to ovipositing adult at 15, 20, 23 and 25°C were 45.8, 32, 19.8 and 17.7 days respectively. At 25°C, the eggs took 6.6 days to hatch, the development time for larvae plus the two nymphal stages was 8.8 days and female adults had a 2.3

day preoviposition period. Adult females had an mean longevity of 17.8 days (at 25°C) and at 23°C laid on average 2.3 eggs per day for 8 days. The mean sex ratio for *T. lintearius* was 1:2.5 (males:females) but can be highly variable in natural conditions due to aggregating behaviour displayed by the females. *T. lintearius* does not appear to display diapausing behaviour and is thought to spend the winter in the form of slowly breeding colonies (Stone, 1986). Colonies of *T. lintearius* move slowly as a group, feeding and web spinning as they travel. Both the eggs and inactive stages are left behind amongst large colonies protected by fine webbing as the active stages in the colony move on (Stone, 1986).

Mites in the genus *Tetranychus* have numerous natural enemies, these include the Chilean predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and species of mite eating ladybirds, *Stethorus* spp. (Coleoptera: Coccinellidae). These predators are known to occur in *T. lintearius* colonies in Tasmania (Ireson *et al.*, 2003).

Another biological control agent for gorse, the gorse seed weevil, *Exapion ulicis* Forster, (Coleoptera: Brentidae), has been present in Australia since 1939 (Evans, 1943). Adult *E. ulicis* feed on gorse foliage and flowers, and oviposit into young, green pods. The larvae of this species then feed and develop within gorse pods destroying a proportion of the seed (Hill *et al.*, 1991). As *T. lintearius* is a foliage feeding agent that produces large colonies protected by fine webbing, it is possible that *T. lintearius* may interfere with *E. ulicis* oviposition on young green pods.

The major aim of this study was to quantify the damage caused by *T. lintearius* on the growth and reproductive capability of gorse. As *T. lintearius* may interfere with *E. ulicis* oviposition and as predators of *T. lintearius* are present in Australia, additional



objectives were to (a) test whether the presence of *T. lintearius* affects numbers of and the subsequent damage caused by the seed feeding biocontrol agent *E. ulicis* and (b) monitor *T. lintearius* colonies for the presence of predators. The use of *T. lintearius* as a biological agent for gorse in Australia, the interaction between agents and the possible impact of predators are discussed.

## 2.2 Materials and Methods

This study was conducted over a period of 30 months. The timing of activities involved in the study are presented in Figure 2.1.

### 2.2.1 Experimental design and trial establishment

The trial was located on a sheep grazing property near Bothwell, Tasmania (42° 22' S, 147° 00' E). The site chosen was approximately 100 m by 20 m, fenced off from stock and situated adjacent to a drainage channel. Gorse was growing amongst semi-improved pasture consisting of a mix of native grasses and introduced grass and clover species. A fire had previously burned the site and the gorse plants were approximately three years of age having regrown from stumps or regenerated from seed.

Twenty similar sized gorse plants, between 1-1.5 m high and 1-2 m wide, were chosen from within the site. The experiment was set up in a completely randomised design, half (10) of the plants were allocated to be treated with *T. lintearius*, the remaining plants served as untreated controls.

To obtain starting points for growth measurements, 50 shoots of the current season's growth were haphazardly chosen within each plant and securely tagged with surveyors tape 15 cm from the growing tip. Each of the marked shoots on each plant were randomly allocated to one of five harvest dates (10 shoots per plant for each harvest date). The shoots chosen for the final harvest were also used for monitoring of *T. lintearius* infestation levels throughout the experiment.

The *T. lintearius* treatment was applied to the gorse plants in late autumn (Fig. 2.1) by introducing four colonies (consisting of approximately 750 mites each) of field

	2001				2002				2003													
	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr											
Activity	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	Comments
Trial establishment																					Bushes chosen and 50 shoots tagged on each bush	
<i>Tl</i> treatment																					4 colonies per bush introduced	
Harvest and assessment		a			a								a								b	
Miticide applications on controls																					c	Controls sprayed with propargite to runoff
Monitoring for <i>Tl</i> and predators																						10 shoots - <i>Tl</i> , whole bushes - predators

*Specific dates of activities:*

Trial establishment - 24/05/2001.

*Tl* treatment – 31/05/2001.

Harvest and assessment - 24/05/2001, 24/08/2001, 1/03/2002, 13/11/2002, 18/11/2003.

Miticide applications on controls – 23/01/2002, 21/03/2002, 27/05/2002, 17/09/2002, 6/01/2003, 5/03/2003, 20/05/2003.

Monitoring for *Tl* and predators - 24/05/2001, 23/07/2001, 21/11/2001, 10/01/2002, 12/03/2002, 22/05/2002, 5/08/2002, 25/09/2002, 7/11/2002, 6/01/2003, 5/03/2003, 16/05/2003, 18/07/2003, 26/09/2003, 18/11/2003.

**Figure 2.1.** Time line of methods used in the assessment of the impact of *Tetranychus lintearius* (*Tl*) on the growth and development of gorse (*Ulex europaeus*) between autumn 2001 and spring 2003. Aut = autumn, Win = winter, Spr = spring, Sum = summer; letters below the seasons represent the months of the year. Codes: (a) Dry weights of vegetative growth only, (b) Dry weights of vegetative growth plus flowers and pods and the gorse seed weevil assessment, (c) Dry weights of vegetative growth plus flowers and pods.

collected *T. lintearius* of mixed stages to each plant. These colonies were positioned next to healthy green foliage at roughly equal distances apart. Colonies were collected from a field site near Jericho, Tasmania (42° 22' S, 147° 16' E). *T. lintearius* was known to be well established at this site and predators were not known to have established (J.E. Ireson, unpublished data). In addition, each colony was inspected with a dissecting microscope at 15× magnification for approximately one minute to reduce the possibility of accidental predator introduction.

To reduce *T. lintearius* damage on control plants, propargite (a.i. 300g/kg; 2-(4-*tert*-butylphenoxy)cyclohexyl prop-2-ynyl sulfite) (Omite<sup>®</sup> WP, Roberts Ltd., Hobart), a mite specific predominantly contact miticide with a long residual activity (Tomlin, 1994), was applied on seven occasions (Fig. 2.1) at a rate of 1g/L. This miticide was applied using a motorised Stihl (model SR 400) backpack sprayer. Controls were sprayed to run-off but no sprays were applied to *T. lintearius* treated plants.

### 2.2.2 Impact of *T. lintearius*

The first of the group of 10 marked shoots to be harvested were collected seven days prior to the application of the *T. lintearius* treatment. The remaining four collections (10 shoots/harvest) were conducted 3, 10, 18 and 30 months following treatment (Fig. 2.1). At each harvest date, branches were cut off at the original surveyors tape tag and transported to the laboratory where further assessment took place.

On the two spring harvest dates when the gorse at the trial site was flowering (Fig. 2.1), all flowers and green pods were counted on each harvested branch. Flowers were counted when petals were visible through the sepals but no pod was visible. Green

Pods were counted when the tip of pod was above the sepals and more than 50% of the pod was green in colour and less than 50% of the pod was black/brown in colour.

On each harvest date, shoot material (from all harvests) and flowers and pods (from the spring harvests) were oven dried at 70°C for 3 days then weighed.

To assess the impact of *T. lintearius*, the mean ( $\pm$  SE) shoot dry weight for control and *T. lintearius* treated plants for each harvest date and a percentage reduction due to *T. lintearius* were calculated. The impact of *T. lintearius* on shoot dry weight of gorse was assessed using a repeated measures ANOVA on untransformed data.

As the miticide treatment was not 100% effective at eradicating *T. lintearius* from control plants, particularly in the final 12 months of the trial (Fig. 2.3), some damage to control plants did occur. To give an estimate of the potential difference between control and *T. lintearius* treated plants as if no damage had occurred to controls, the number of times following *T. lintearius* application that each of the ten final harvest shoots per plant were not infested with *T. lintearius* was calculated as a proportion ( $n = 140$  per plant) from monitoring data (see section 2.2.3) and used as a damage index. A linear regression was conducted on the proportion of non-infested shoots per plant (x axis) and mean final shoot dry weight (y axis) with a point for each plant in the control and *T. lintearius* treatments. This yielded an equation in the form:  $y = a + bx$ , where  $y$  = mean shoot dry weight,  $x$  = proportion of non-infested shoots,  $b$  = the slope of the regression line and  $a$  = y intercept. A predicted control (no mite) mean shoot dry weight was calculated by solving the equation for  $y$  when  $x = 1$ .

Total flower and green pod production per plant was highly variable across both *T. lintearius* treated plants and untreated controls. Hence, non-parametric Mann-Whitney tests were used to compare flower and green pod production per plant in both the

2002 and 2003 seasons. A further check was made on the 2003 flower and green pod season to see whether *T. lintearius* damage to controls in 2003 influenced this result. This found that neither the number of flowers or of green pods per plant in 2003 were significantly correlated to the proportion of shoots infested with *T. lintearius* per plant in 2003 (flowers:  $r^2 = 0.01$ ,  $F_{1,17} = 0.17$ ,  $P = 0.69$ ; pods:  $r^2 = 0.025$ ,  $F_{1,37} = 0.43$ ,  $P = 0.52$ ). To determine if the differences in flower and green pod production between the 2002 and 2003 seasons was significant between *T. lintearius* treated plants and untreated controls, the changes in the number of flowers and green pods (2003 - 2002) were calculated for each plant and compared using the non-parametric Mann-Whitney test. Finally, to determine if there was a difference in the numbers of flowers or green pods produced between the 2002 and 2003 seasons, a paired sample t-test was conducted.

### 2.2.3 Monitoring *T. lintearius* and predators

To determine the period of *T. lintearius* activity, monitoring was conducted during the course of the trial approximately every 2 months (Fig. 2.1). On each monitoring date, the same 10 shoots on each control and *T. lintearius* treated plant previously chosen for monitoring and the final harvest were inspected for approximately 30 seconds each with a hand lens (6× magnification). The presence or absence of *T. lintearius* was recorded.

To determine the period of predator activity, each plant that had *T. lintearius* present was inspected with the hand lens for approximately 3 minutes to check for the presence of two predators, *Phytoseiulus persimilis* and *Stethorus* spp. For this predator assessment, the shoots chosen for the assessment of *T. lintearius* were not

necessarily inspected, but instead particular attention was paid to areas on the plant where *T. lintearius* eggs and juveniles were aggregated.

#### 2.2.4 Interaction with *E. ulicis*

To assess whether *T. lintearius* had an interference effect on *E. ulicis* populations, *E. ulicis* numbers were compared in gorse pods between *T. lintearius* treated plants and the untreated controls on the spring 2002 harvest prior to any confounding invasion of the controls by *T. lintearius* (Fig. 2.1). On shoots that had pods present, up to five green pods per shoot were randomly selected. These pods were dissected and scored as infested (*E. ulicis* present) or uninfested (*E. ulicis* not present). The number of undamaged and damaged seed was recorded as well as the number of eggs and larvae of *E. ulicis* found within them.

The interaction of *T. lintearius* with *E. ulicis* was assessed using Mann-Whitney tests by comparing the number of eggs, larvae and the proportion of damaged seed in green pods per plant between *T. lintearius* treated plants and untreated controls. To further examine the oviposition behaviour of *E. ulicis*, regression analyses were used. Firstly, the relationship between the number of eggs of *E. ulicis* within a pod and the number of seed within a pod was examined. Secondly, the relationship between the number of pods per plant and the percentage of pods on a plant that were infested by *E. ulicis* was investigated.

## 2.3 Results

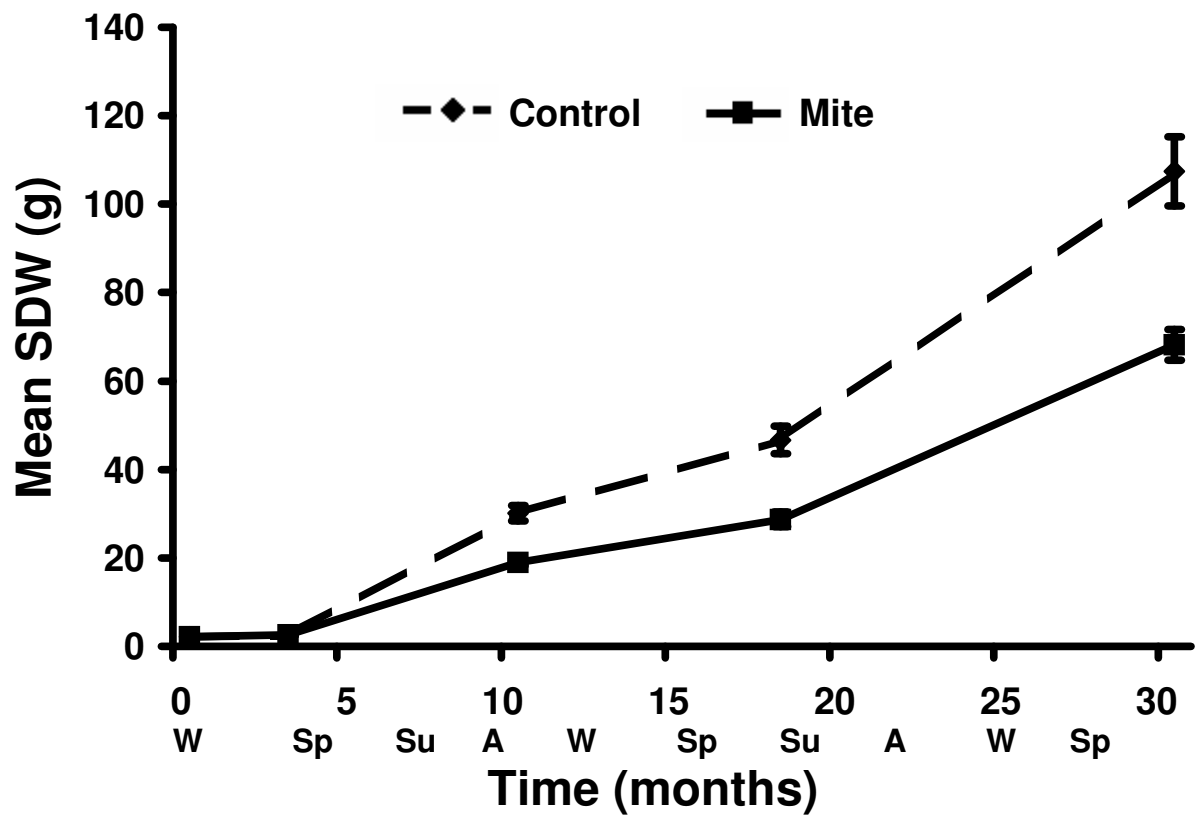
### 2.3.1 Impact of *T. lintearius*.

No death of any of the *T. lintearius* treated plants occurred during the study. One of the control plants died suddenly in autumn 2003, however, this was attributed to localised soil waterlogging. As this plant appeared healthy during most of the experiment, the shoot dry weight data from the first four harvests were used in the analysis.

The *T. lintearius* treatment significantly reduced the dry weight of harvested shoots ( $F_{1,18} = 6.9$ ,  $P = 0.017$ ; Fig 2.2). This difference did not become evident until autumn 2002, 10 months from the start of the experiment. At this time the mean dry weight of *T. lintearius* damaged shoots (19.0 g) was 37% lower than control shoots (30.1 g). At 17 months from the start of the experiment the dry weight of *T. lintearius* damaged shoots was 38% lower than control shoots. Similarly, in the final assessment 30 months from the start of the experiment, the dry weight of *T. lintearius* damaged shoots (68.8 g) was 36% lower than control shoots (107.4 g).

When the proportion of non-infested shoots (which varied between 63-93%) was plotted against the respective mean shoot dry weights the following equation resulted:  $y = 163.91x - 42.204$  ( $r^2 = 0.14$ ,  $F_{1,37} = 2.77$ ,  $P = 0.11$ ). The predicted mean dry weight of the controls if no *T. lintearius* damage had occurred (ie. when  $x = 1$ ) was therefore calculated at 121.72 grams. Using this predicted dry weight, it is evident that the presence of *T. lintearius* would have caused an even greater reduction in dry matter production of approximately 44% if damage to controls could have been prevented.



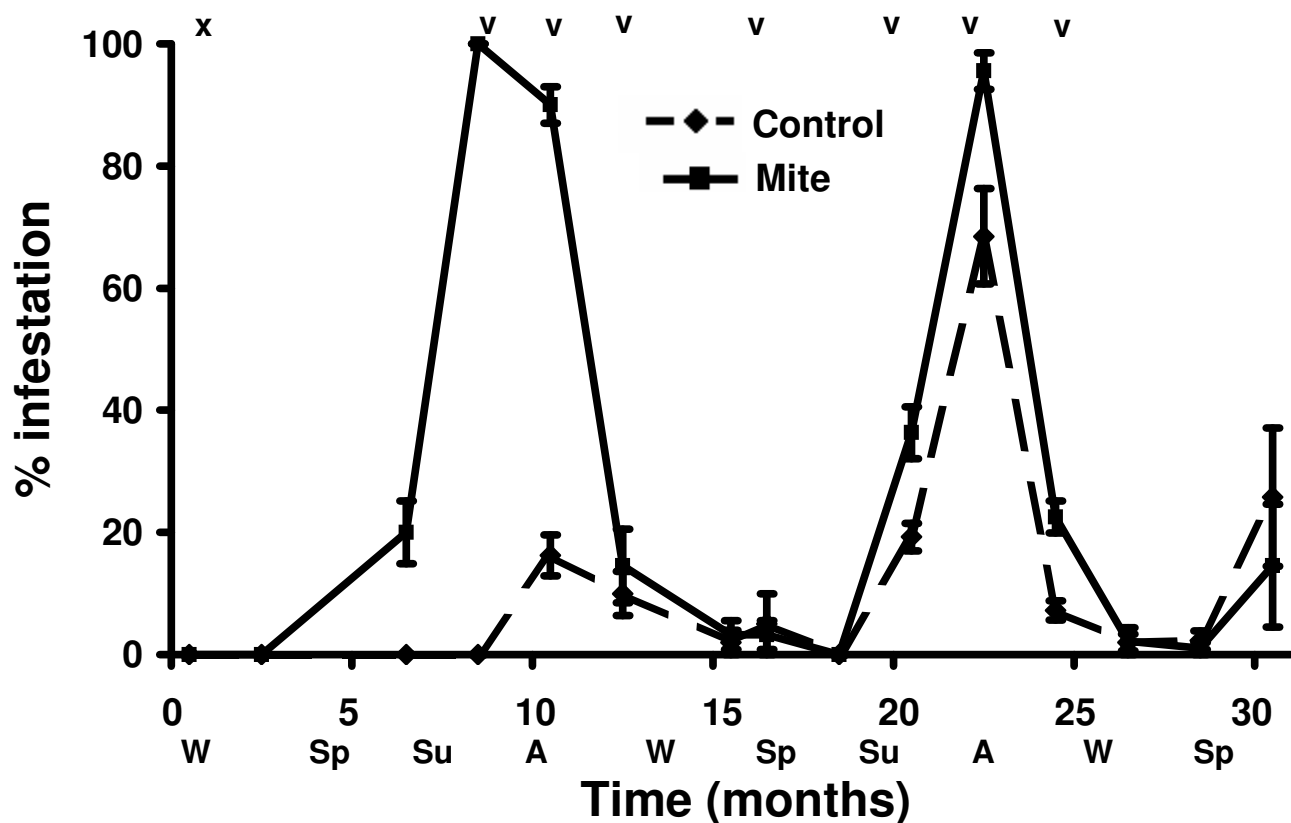


**Figure 2.2.** Mean ( $\pm$ SE) shoot dry weight (SDW) (grams) of gorse plants treated with *T. lintearius* (mite) and a miticide treated control between May 2001 and November 2003. Each treatment/date combination consisted of 10 subsample shoots within 10 replicate plants. W = Winter, Sp = Spring, Su = Summer, A = Autumn.

Flower and green pod production was highly variable between plants. In spring 2002, there were significantly more flowers on shoots harvested from *T. lintearius* treated plants than control plants ( $U_{1,10,10} = 80$ ,  $P = 0.023$ ) but no significant difference in spring 2003 ( $U_{1,10,9} = 41$ ,  $P = 0.74$ ). The number of green pods ( $U_{1,10,10} = 61.5$ ,  $P = 0.38$ ) ( $U_{1,10,10} = 34$ ,  $P = 0.37$ ) showed no significant difference. There was also no significant difference between control and *T. lintearius* treated plant in the change each plant had in the level of flower or of green pod production between the two seasons (flowers:  $U_{1,10,9} = 35$ ,  $P = 0.41$ ; green pods:  $U_{1,10,9} = 42$ ,  $P = 0.81$ ). There was also no significant difference between seasons (2002 and 2003) in the number of flowers or green pods on plants irrespective of treatment (flowers: paired  $t_{18} = 0.047$ ,  $P = 0.96$ ; green pods: paired  $t_{18} = 1.09$ ,  $P = 0.29$ ). Flower production was  $8.0 \pm 5.0$  flowers per shoot in 2002 and  $8.7 \pm 3.5$  flowers per shoot in 2003. Pod production was  $2.42 \pm 0.71$  pods per shoot in 2002 and  $3.78 \pm 1.13$  pods per shoot in 2003.

### 2.3.2 Monitoring *T. lintearius* and predators

*T. lintearius* was introduced in late autumn 2001 and by summer 2002 had colonised 100% of the monitored shoots on *T. lintearius* treated plants (Fig. 2.3). However, the percentage infestation fell to less than 20% by winter 2002 then rose sharply in late spring until approaching 100% again by early autumn 2003. On control plants, no *T. lintearius* infestation was recorded until early autumn 2002 when on average 16% of shoots per plant were infested (Fig. 2.3). In late autumn and winter the infestation levels of control plants showed a similar seasonal decline as recorded on the treated plants. In late spring 2002 and summer 2003, despite a miticide spraying regime, the percentage infestation increased on the controls until a peak of 69% in early autumn 2003. In autumn and spring 2003, there were similar levels of *T. lintearius* attack and



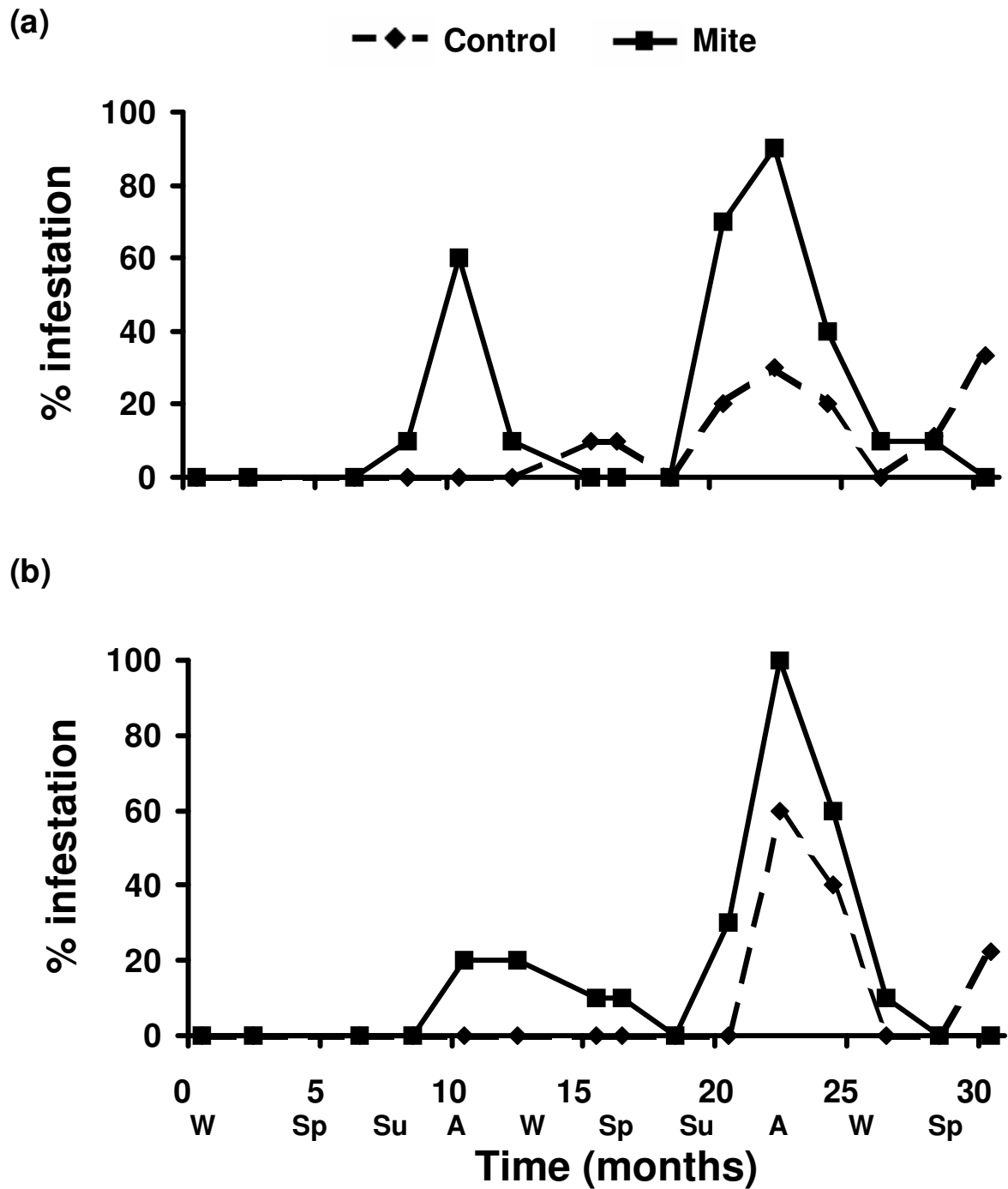
**Figure 2.3.** Mean ( $\pm$ SE) *T. lintearius* infestation levels per gorse plant treated with *T. lintearius* (mite) and a miticide treated control between May 2001 and November 2003. Each treatment/date combination consisted of 10 subsample shoots within 10 replicate plants that were assessed for *T. lintearius*. **x** = time of *T. lintearius* introduction. **v** = time of miticide applications, W = Winter, Sp = Spring, Su = Summer, A = Autumn.

the distinctive bleaching damage symptoms were observed on both the treated and control plants.

Both predators, *Phytoseiulus persimilis* and *Stethorus* sp., were found within *T. lintearius* colonies at the trial site during the course of this study. *Stethorus* sp. was the first predator to be observed in January 2002, less than eight months after the initial release of *T. lintearius*. This species infested a maximum of 60% of *T. lintearius* treated plants in March 2002 then a peak of 90% in March 2003 (Fig. 2.4a). *Stethorus* sp. was similarly present on control plants but appeared later (winter 2002) and was mainly at lower levels. *P. persimilis* was first observed in March 2002, approximately 10 months after the initial release of *T. lintearius*. In that season it infested a maximum of 20% of *T. lintearius* treated plants. In the following season the levels of infestation increased, *P. persimilis* infested a maximum of 100% of *T. lintearius* treated plants in March 2003 (Fig. 2.4b). *P. persimilis* was similarly present on control plants but appeared later (summer 2003) and was at lower levels (60%).

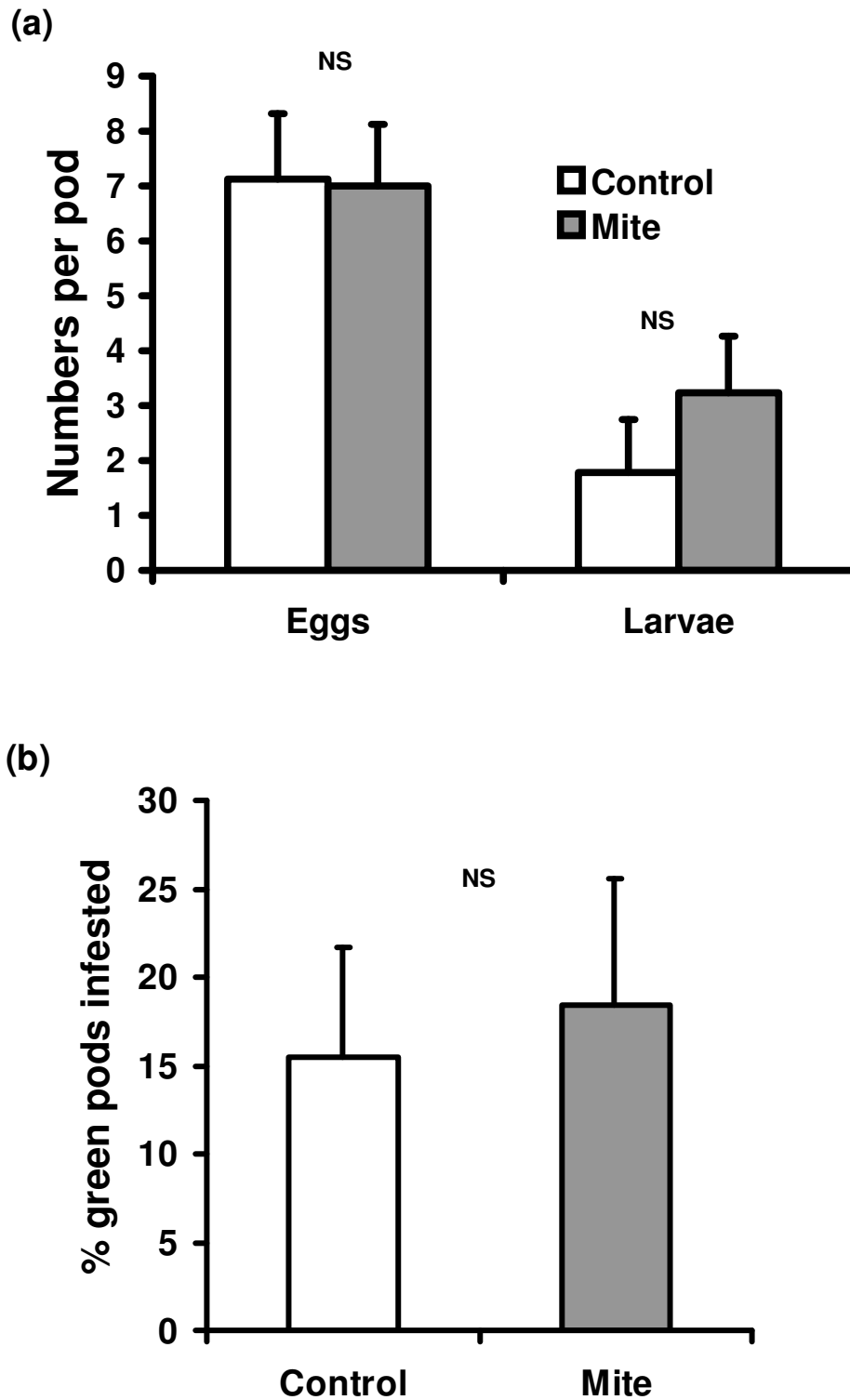
### 2.3.3 Interaction with *E. ulicis*

The *T. lintearius* treatment had no significant effect on the numbers of *E. ulicis* eggs ( $U_{1,6,9} = 31$ ,  $P = 0.64$ ) or larvae ( $U_{1,6,9} = 0.19$ ,  $P = 0.34$ ) in pods compared to the miticide treated control (Fig 2.5a). There was a mean of 6.6 (range 0-40) and 5.5 (range 0-31) eggs per pod and only about half the numbers of larvae than eggs, with a mean of 2.7 (range 0-19) and 3.3 (range 0-22) larvae per pod in control and *T. lintearius* treated plants respectively. Similarly, the *T. lintearius* treatment had no significant effect on the proportion of seed in green pods damaged by *E. ulicis* larvae compared to the control ( $U_{1,6,9} = 0.23$ ,  $P = 0.63$ ). Means of 22.7% and 23.5% seed



**Figure 2.4.** Percentage of gorse plants treated with *T. lintearius* (mite) and a miticide treated control between May 2001 and November 2003 that were also infested with predators (a) *Stethorus* sp. and (b) *Phytoseiulus persimilis*. 10 bushes for each treatment were inspected with a  $\times 6$  hand lens for approximately 3 minutes to determine predator presence. W = Winter, Sp = Spring, Su = Summer, A = Autumn.

Fig. 2.4



**Figure 2.5.** (a) Mean ( $\pm$ SE) number of *Exapion ulicis* eggs and larvae and (b) mean ( $\pm$ SE) percentage of seed in green pods damaged by *E. ulicis* larvae from gorse plants treated with *T. lintearius* (mite) and a miticide treated control. Each treatment consisted of a maximum of 5 green pods from each of 10 subsample shoots within 10 replicate plants (NS =  $P > 0.05$ ; Mann-Whitney test).

damage were recorded on control and *T. lintearius* treated plants respectively (Fig. 2.5b).

The number of *E. ulicis* eggs per pod and the number of seeds in a pod were significantly correlated (eggs:  $r^2 = 0.060$ ,  $F_{1,140} = 8.95$ ,  $P = 0.0033$ ). However, as the percentage of pods on a plant that were infested by *E. ulicis* was not significantly correlated to the number of pods on plants ( $r^2 = 0.0041$ ,  $F_{1,13} = 0.05$ ,  $P = 0.82$ ), there is no evidence that *E. ulicis* is acting in a density dependent manner.

## 2.4 Discussion

The significant reduction in dry matter production caused by *T. lintearius* in this study indicates that this species has the potential to be a useful agent. Preliminary studies by others on the impact of *T. lintearius* were conducted over different time frames, under different environmental conditions using different assessments of growth. Despite these differences, studies in New Zealand (Fowler and Griffin, 1995; T.R. Partridge unpublished data cited by Richardson and Hill 1998; Landcare Research, unpublished data) and in Oregon, USA (Rice, 2004) also found that *T. lintearius* can cause significant reductions in growth. In this study, the measured reduction in growth of 36% on the *T. lintearius* treated plants after two and a half years could have been greater (ie. approximately 44%) if it had been possible to prevent *T. lintearius* damage to the controls.

Although the death of gorse plants attributed to *T. lintearius* attack was observed by Zwolfer (1963), *T. lintearius* did not cause the death of any gorse plants in this study. However, the measured growth reduction of 36% (or greater) could benefit attempts to re-establish a gorse infested area with more desirable plant species. Pasture species can compete strongly with gorse seedlings (Ivens, 1979; Ivens and Mlowe, 1980) so pasture competition could be an important component of an integrated control strategy incorporating biological control agents. For instance, pasture competition was shown to reduce both the survival and shoot dry weight of gorse seedlings when combined with another biocontrol agent of gorse, *Sericothrips staphylinus* (Haliday) (Thysanoptera: Thripidae), in a glasshouse experiment (Davies *et al.*, 2005, see Chapter 5).



Herbivory and competition are two of the main forces shaping the composition of plant communities (del-Val and Crawley, 2005). It is widely accepted that even small reductions in growth of one plant species can result in major shifts in the competitive balance of plant communities (Crawley, 1989). Therefore the reduction in growth caused by *T. lintearius* may reduce the competitive ability of gorse and be beneficial to more desirable plant species by enabling them to compete with gorse more effectively. Furthermore, the feeding of *T. lintearius* and other biological control agents could have an effect on the longevity of gorse, which can exist in some plant stands for up to 33 years (Richardson and Hill, 1998).

Although *T. lintearius* had no apparent impact on pod production such an effect may take a more extended time period to develop. The effect of *T. lintearius* on flowering was not clear in this study but flower production was enormously variable between plants irrespective of treatment. In spring 2002, 18 months after *T. lintearius* introduction there was a increase in flowering due to *T. lintearius*. However, in spring 2003, 30 months after introduction, the mean numbers of flowers on the *T. lintearius* treated plants was not significantly different to the control plants. Furthermore, there was no correlation between *T. lintearius* damage levels and either flower or pod production in 2003. The allocation of resources within a plant can be dramatically altered following herbivore damage, including either decreasing or increasing the production of flowers or fruit (Trumble *et al.*, 1993). However, little evidence either way was determined in this study and further studies with larger sample sizes to overcome flowering variability would be required to determine if flowering and pod production of gorse is affected by *T. lintearius*.

This study assessed the impact of *T. lintearius* on gorse during the first 2.5 years following its introduction to a site. However, on an ecological time scale, this is a relatively short time period. *T. lintearius* populations and the subsequent damage to gorse may fluctuate considerably over more extended time periods. Tetranychid mite populations are notoriously patchy in time and space due to ecological factors such as temperature, exposure to rainfall, host plant quality and natural enemies (Sabelis, 1985). This patchy distribution of *T. lintearius* populations and the subsequent localised damage to gorse has been observed at Tasmanian field sites (J. E. Ireson, unpublished data). Following release and initial establishment, *T. lintearius* can cause severe damage to gorse in localised areas. Eventually, however, numbers start to decrease probably as a result of predation or migration triggered by the presence of predators, colony size and the decline in food quality (Ireson *et al.*, 2004).

Determination of the impact of the predators' *Stethorus* sp. and *P. persimilis*, which were recorded eight and ten months into the study respectively, and were active on *T. lintearius* colonies throughout the site, was beyond the scope of this study. However, a predator exclusion study in the USA (Pratt *et al.*, 2003) found that predation by *P. persimilis* significantly reduced the volume and number of *T. lintearius* colonies. It is therefore probable that predation would ultimately have caused a significant decline in the numbers and effectiveness of *T. lintearius* at the Bothwell site after the completion of the experiment. Studies in Victoria and Tasmania have shown that predation of *T. lintearius* by *Stethorus* sp. and *P. persimilis* is already widespread (Ireson *et al.*, 2003) and probably a key factor in restricting its usefulness as a biological control agent. Furthermore, a controlled temperature development study in Tasmania as part of this investigation (Davies *et al.*, 2004, see Chapter 3), found that *P. persimilis* will complete its pre adult development in less than half the time of *T.*

*linterarius*. Interestingly, although *Stethorus* sp. has been recorded in gorse infestations in the high rainfall areas of the West Coast and parts of the North West of Tasmania, *P. persimilis* has not (J. E. Ireson, pers. comm.). However, in these locations, high rainfall may limit the establishment of *T. linterarius* populations more than predation (Ireson *et al.*, 2003).

Although the results from this study suggest that *T. linterarius* did not negatively interfere with the seed feeding agent *E. ulicis*, such an interference may only occur in certain circumstances. For instance, *E. ulicis* only oviposits into young green gorse pods in spring (Hill *et al.*, 1991), but in this study *T. linterarius* activity peaked in late summer and autumn. It is possible that a mild, dry winter could allow a build up of larger numbers of *T. linterarius*, resulting in extensive webbing on the plants during the spring and early summer thus coinciding with gorse flowering. Perhaps an interference effect could be observed under these circumstances.

Although predation and other factors may be restricting the efficacy of *T. linterarius*, its usefulness must be considered as part of the overall biological control program for gorse involving established and additional biological control agents. If *T. linterarius* can reduce the growth rate of gorse while not interfering with other agents, then it could be a useful component of a guild of agents that attack gorse at different stages of its lifecycle. If these agents are successful at reducing the vigour and competitiveness of gorse, biological control could become an important long-term component of a successful integrated management strategy.

### **Chapter 3. The development time of two populations of *Phytoseiulus persimilis*, on diets of the two spotted mite, *Tetranychus urticae*, and the gorse spider mite, *Tetranychus lintearius*, and its implications for the biological control of gorse, *Ulex europaeus*.**

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#### **Abstract**

The development time and mortality were determined for two different populations of the predatory phytoseiid mite, *Phytoseiulus persimilis*, at 14°C and 24°C, on diets of *Tetranychus urticae* and *T. lintearius*. One population was obtained from a commercial insectary where it was reared on *T. urticae* and the other population was collected from gorse plants at a field site where it was feeding on *T. lintearius*. At 14°C, the mean development time from egg to adult for *P. persimilis* was 17.8 days. Diet had no significant effect on the development time and there was also no significant difference in development time between the two populations. At 24°C, the mean development time from egg to adult was 5.8 days and again there was no significant difference between the development time of the two populations. However, diet did have a significant effect on development time at 24°C, with both populations of *P. persimilis* completing their development almost half a day earlier when fed on a diet of *T. lintearius* (5.6 days) compared to a diet of *T. urticae* (6.0 days). For both populations, the mortality of *P. persimilis* was significantly higher on a diet of *T. lintearius* than it was on a diet of *T. urticae*. There was no significant difference in mortality on either diet between the two populations of *P. persimilis* nor was there a significant effect on mortality due to temperature. As no difference in either mortality or development time could be detected between the two populations of *P. persimilis*, there is no evidence that the two populations are different strains. Predator/prey generation time ratios were calculated between *P. persimilis* and both *T. urticae* and *T. lintearius* using data from this and other studies. These results are discussed with regard to the generation time ratio hypothesis, indicating that *P. persimilis* is likely to have a negative impact on *T. lintearius* and therefore reduce its effectiveness as a biological control agent for gorse.

### 3.1 Introduction

Host specific phytophagous arthropods are often introduced as biological control agents for invasive weeds. Natural enemies, including predatory arthropods, play a key role in regulating populations of many phytophagous arthropods (Berryman, 1999) and are extensively utilised for the biological control of agricultural and horticultural pests. However in weed biological control, where large populations of damaging phytophagous arthropods are desired, natural enemies may reduce the population size and therefore the effectiveness of weed biological control agents (Goeden and Louda, 1976; McFayden and Spafford-Jacob, 2004).

Gorse, *Ulex europaeus* L. (Fabaceae), is a leguminous European woody shrub that has become a serious weed in many temperate regions of the world. In Australia, gorse is a weed of national significance (Thorp, 1999) seriously affecting agricultural and environmentally significant regions in south-eastern Australia (see Chapter 1). As part of an integrated management strategy, a guild of host specific biological control agents are currently being introduced to gorse in Australia (Ireson *et al.*, 2004).

The gorse spider mite, *Tetranychus lintearius* Dufour (Acari: Tetranychidae), was introduced to Australia from New Zealand and released in 1998. This species is now established in Tasmania, Victoria and New South Wales (Ireson *et al.*, 2004). Its impact was demonstrated in a field study conducted in Tasmania (see Chapter 2). In this study the presence of *T. lintearius* colonies significantly reduced the dry weight of foliage on three year old gorse bushes by approximately 37% over a period of 2.5 years from the time of initial infestation.

Natural enemies that have the potential to reduce the efficacy of *T. lintearius* have been identified in Australian surveys (Ireson *et al.*, 2003). One of these was the

introduced Chilean predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), which has been shown to significantly reduce the size of *T. lintearius* colonies in predator exclusion studies in the USA (Pratt *et al.*, 2003). This species is a specialist predator of Tetranychid mites and is capable of reducing pest *Tetranychus* spp. populations below economically damaging levels (McMurtry and Croft, 1997). As it is so effective, *P. persimilis* is available from biocontrol companies in many parts of the world including Australia (eg. [www.beneficialbugs.com.au](http://www.beneficialbugs.com.au)). It is commonly used as a biocontrol agent of a polyphagous pest, the two spotted mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and other pest *Tetranychus* spp. in a variety of crops (McMurtry and Croft, 1997).

The generation time ratio hypothesis (Dixon *et al.*, 1997) predicts that predators will have little impact on the abundance of their prey in ecological systems where predators have longer generation times than their prey. Conversely, the predators may have a significant impact on the abundance of their prey in systems where predators have shorter generation times than their prey. Therefore, predators with a smaller predator: prey generation time ratio ( $<1$ ) may be more effective biological control agents of a particular pest arthropod than predators with a larger predator: prey generation time ratio ( $>1$ ) (Dixon *et al.*, 1997). Diet can have an influence on the development time of predators.

Studies have previously been conducted on the development of *P. persimilis* on diets of pest *Tetranychus* spp. including *T. urticae* (eg. Galazzi and Nicoli, 1996), *T. kanzawai* (Hamamura *et al.* 1976) and *T. pacificus* (Perring and Lackey, 1989). In a Spanish study, Escudero and Ferragut (2005) compared life history characteristics, including development time and survival, of *P. persimilis* when fed four different

*Tetranychus* species. Predator performance on the different *Tetranychus* species varied and was particularly poor when *P. persimilis* was fed on a diet of *T. evansi*. The authors suggest that this may result in a poor ability of *P. persimilis* to control *T. evansi* in a cropping situation. The performance, including development time from egg to adult and survival, was greatly improved when *P. persimilis* was fed on the other *Tetranychus* species in their study (*T. urticae*, *T. turkestanii* and *T. ludeni*).

The development time of predators can also vary between strains that may originate from different localities. Perring and Lackey (1989) established that a strain of *P. persimilis* from Israel developed from egg to adult almost half a day earlier than a strain from California, USA at 26.7°C and 73% RH. In other studies on the development of *P. persimilis*, significant differences were also found between the development time of different strains of *P. persimilis* originating from Great Britain, Southern Italy and Northern Italy (Galazzi and Nicoli, 1996) and Czechoslovakia and Ukraine (Praslicka and Uhlik, 1999).

*P. persimilis* seems to have adapted well to the cool temperate climate in the midlands of Tasmania and is now using *T. lintearius* as part of its diet (Ireson *et al.*, 2003). It is therefore possible that the performance of *P. persimilis* collected from the field in Tasmanian may significantly differ from *P. persimilis* that is commercially available from biocontrol companies. *P. persimilis* from the midlands of Tasmania will have undergone multiple generations at cooler temperatures whereas *P. persimilis* from biocontrol companies are continually exposed to the warmer conditions of glasshouse rearing. A strain is defined as a "population with morphological, physiological, or behavioural traits that distinguish them from other con-specific populations" (Clarke and Walter, 1995). Therefore, if there were differences in the development of the two

populations of *P. persimilis*, this would provide evidence that the Tasmanian field population is a separate strain to the population sourced from a biocontrol company.

The aim of this study was to determine if the development time and mortality of two populations of *P. persimilis* differ when reared on diets of *T. urticae* and *T. lintearius*. This was undertaken to confirm that *T. lintearius* is a suitable food source for *P. persimilis*, and to test if the Tasmanian field population of *P. persimilis* is a distinct strain, differentiated by development time, compared to a commercially available glasshouse reared population. Finally, the development times of *P. persimilis* were compared to the development times of *T. urticae* and *T. lintearius* in order to compare the generation time ratios of predator and prey as an index of their effectiveness on these prey diets. The implication of these results on the effectiveness of *T. lintearius* as a biological control for gorse is discussed.



## 3.2 Materials and Methods

All rearing and development experiments were conducted using facilities at New Town Research Laboratories (NTRL), located near Hobart, Tasmania, Australia.

### 3.2.1 Production of prey eggs

Eggs of *T. urticae* were produced for development experiments on bean plants. Young potted bean, *Phaseolus vulgaris* 'Redland Pioneer' (sourced from Yates, [www.yates.com.au](http://www.yates.com.au)), were grown from seed until approximately 20cm in height. A starter plant was initially infested with approximately 150 *T. urticae* of mixed stages collected from white clover, *Trifolium repens* L. (Fabaceae), growing as a weed on the ground in a polytunnel at NTRL. Additional plants were placed adjacent to the infested plant as required until approximately 40 plants were infested. The plants were maintained for approximately eight weeks from the time of initial infestation in a glasshouse with supplementary heating until high levels of *T. urticae* damage occurred. Additional uninfested *P. vulgaris* plants were placed within infested plants for 24 hours to allow ambulatory dispersal of adult mites. These were removed and placed in a controlled temperature cabinet at 23°C and a 16 hour photoperiod for approximately 24 hours to allow egg laying to take place.

After this period, approximately 25 heavily infested leaves were removed and placed in a 200mL beaker containing 150 mL of a 0.5% sodium hypochlorite solution and stirred briskly with a glass rod. The leaves were removed from the beaker and the solution containing eggs was poured through a synthetic fabric (60 micron monomesh, Dustcotech DHH consultants Pty Ltd, Bassendean, Western Australia) and carefully washed with distilled water. The filtered eggs were then removed with a fine sable hair brush. Eggs were stored on small (3 cm × 3 cm) pieces of filter paper

on a petri dish for no more than 48 hours in a 5°C refrigerator until required in experiments.

Eggs of *T. lintearius* were produced for development experiments on young gorse shoots. Fresh gorse stems were pruned from greenhouse grown gorse plants. These were trimmed to approximately 20 cm in length, all branches were cut off the lower 10 cm and branches on the upper 10 cm were lightly pruned so that a cylinder of gorse foliage, approximately 2 cm in diameter by 10 cm in length, was produced. The lower 10 cm of each cutting was inserted into water through a 4 mm hole in the centre of a screw top lid on a plastic vial 8 cm high and 3 cm in diameter. The cuttings (20 in total) were allowed to air dry and approximately 300 adult *T. lintearius* cultured on potted gorse plants (Ireson *et al.*, 1999) in a glasshouse at around 20°C were added to each cutting. The tubes containing the cuttings with added mites were placed in a controlled temperature cabinet at 23°C and a 16 hour photoperiod for approximately 24 hours to allow egg laying to take place. The gorse cuttings were then removed from the vials of water and briskly rotated in a 200mL beaker containing 150 mL of a 0.5% sodium hypochlorite solution. This solution, which now contained the eggs, was treated and stored until use using the same methodology as for *T. urticae*.

### 3.2.2 Predator populations and rearing

The two populations of *P. persimilis* used in this experiment were collected from different sources feeding on different *Tetranychus* species. The Tasmanian population (Tas *P. persimilis*) was collected from a field site at Stonehenge, Tasmania (42° 24' S, 147° 37' E), from within *T. lintearius* colonies on gorse. The Tas *P. persimilis* population had been observed feeding on *T. lintearius* for more than 18 months at the time this experiment was initiated (Ireson *et al.*, 2003). Stonehenge is in an inland

region at 300 m above sea level and the long term (1882-2004) record of annual temperature of the nearest weather station (Oatlands) ranges between a daily mean minimum temperature of 5°C and a daily mean maximum temperature of 15.4°C (Bureau of Meteorology 1995). The second population was supplied by the 'Beneficial Bugs Co.' ([www.beneficialbugs.com.au](http://www.beneficialbugs.com.au)) and had been reared on *T. urticae* colonies on bean (*Phaseolus vulgaris*) at Richmond, NSW (NSW *P. persimilis*). This population was reared at approximately 25°C in insectary conditions.

Eggs of both Tas and NSW *P. persimilis* populations were produced for the development experiment on detached bean leaf arenas. Bean (*Phaseolus vulgaris* 'Redland Pioneer') leaflets were detached from plants and placed upside down onto Petri plates containing 7 g/L water agar with the pedicels bent over and inserted into the agar. Numerous *T. urticae* eggs, collected as previously described, were placed onto each leaf arena. Five adult female *P. persimilis* were then placed onto each arena and these were then placed into a controlled temperature cabinet at 23°C and a 16 hour photoperiod. Arenas were inspected every 2 hours and all *P. persimilis* eggs present were collected and immediately placed into perspex arenas for the development experiment (see next section) with abundant prey eggs.

### 3.2.3 Determination of *P. persimilis* development times

In a factorial experiment, Tas and NSW *P. persimilis* were reared from egg to adult on the two diets (*T. urticae* and *T. lintearius*) in a randomised design. The experiment was conducted in two separate controlled temperature cabinets, which maintained the chosen temperatures (14°C ± 0.7 and 24°C ± 0.7) and a daily photoperiod of 16 hours. At each temperature 40 eggs of each of Tas and NSW *P. persimilis* were used. Diets consisted of abundant eggs of either *T. lintearius* or *T. urticae*, which were reared and

extracted as previously described. Half of each of Tas and NSW *P. persimilis* (20) were allocated to each diet at each temperature.

Rearing was conducted in arenas similar to those described by Perring and Lackey (1989). Arenas were constructed from  $200 \times 50 \times 6$  mm pieces of perspex. Fourteen tapered holes with diameters of 13 mm on top and 7 mm on the bottom were drilled in each piece of perspex to form the arenas. Arenas were sealed to prevent mite escape and allow unimpeded viewing. On the larger top hole, a microscope cover slip with a diameter of 17 mm was fixed to the perspex using a 1:8 mix of Vaseline<sup>®</sup> and beeswax. To allow airflow into the arenas, a synthetic fabric (60 micron monomesh) was fixed onto the smaller bottom hole using the same vasoline/beeswax mix.

One egg of *P. persimilis*, less than two hours of age, was placed into each arena.

Arenas were housed within  $35 \times 27 \times 19$  cm translucent lidded plastic boxes containing 2 litres of saturated NaCl solution, which maintained relative humidity at 75% at both temperatures (Winston and Bates, 1960).

Development times from egg through to adult were determined by counting the number of cast skins in each arena at each observation every 12 hours. Mites that died during or just after a moult were considered to have achieved the more advanced life stage.

#### 3.2.4 Comparison of development of *P. persimilis* and prey *Tetranychus* spp.

Three searches were conducted on the CAB abstracts database between the years 1973 and 2005 to identify studies that have experimentally determined the development times of *P. persimilis*, *T. lintearius* and *T. urticae* at different temperatures. Keywords used were '*Phytoseiulus persimilis* and development and

temperature' for the first search, '*Tetranychus lintearius* development and temperature' for the second search and '*Tetranychus urticae* and development and temperature not predator' for the third search. 'Not predator' was specified in the third search to eliminate the numerous studies on predators of *T. urticae*.

From this literature, the data were pooled from all relevant studies on each species (although there was only one relevant study for *T. lintearius*). Pre-adult development times (in days) of each mite species were collated. Linear regressions were conducted for temperature vs. development rate (1/development time in days) for each species (Data used for conducting the regressions is displayed in Figures 3.1 a, b and c and the full data set is provided in Appendix 1). Regressions yielded an equation in the form  $y = a + bx$ , where  $y$  = development rate,  $x$  = temperature,  $b$  = the slope of the regression line and  $a$  =  $y$  intercept. The lower development thresholds of the three mite species were estimated by solving the regression equation for  $y$  (development rate) = 0. The number of day degrees required for development from egg to adult for each mite species was estimated by  $1/b$ . Standard errors for day degrees and lower development thresholds were calculated using the methods of Campbell *et al.* (1974).

The generation times (time in days for the development of each species from egg to adult) were determined for all three species at the standardised temperatures of 15°C and 25°C by solving the reciprocal of  $y$  in the above equation when  $x = 15$  and 25 respectively. Standard errors for these predicted generation times were calculated using the methods of Zar (1999). The predator: prey generation time ratios between *P. persimilis*: *T. urticae* and *P. persimilis*: *T. lintearius* were calculated at the temperatures of 15°C and 25°C by dividing the calculated generation times of *P.*

*persimilis* by the calculated generation times of *T. urticae* and *T. lintearius* respectively.

### 3.2.5 Data analysis

Statistical tests for diet and predator population differences were performed using SYSTAT 10th edition. All analyses for both mortality and development time were conducted on the development from the protonymph to adult only as the egg and larval stages of *P. persimilis* do not feed (Schausberger and Croft, 1999). To determine if there were differences in the mortality between Tas and NSW *P. persimilis*, diets, or temperature, data on the mortality of *P. persimilis* from protonymph to adult were subjected to Chi-Square analyses with Yates correction factor. Due to the pseudo-replication between temperatures, the assumption was made in this test that the only variable differing between the two controlled temperature cabinets was temperature. Data on the development time of *P. persimilis* from protonymph to adult were independently subjected to ANOVA for each temperature to test for differences between Tas and NSW *P. persimilis* and diet. Where significant differences were found using ANOVA, means of each combination of Tas and NSW *P. persimilis* on each diet of all life stages were separated using Fishers Least Significant Difference test.

### 3.3 Results

#### 3.3.1 Mortality of *P. persimilis*

There was a significant difference in mortality of both Tas and NSW *P. persimilis* across both temperatures in response to diet. The mortality of *P. persimilis* from protonymph to adult was significantly higher on a diet of *T. lintearius* (16.2 %) than on a diet of *T. urticae* (4.4 %) (Yates corrected  $\chi^2 = 3.9$ ,  $df = 1$ ,  $P = 0.048$ ). The mortality of *P. persimilis* between the protonymph and adult did not, however, significantly differ in response to temperature (Yates corrected  $\chi^2 = 2.7$ ,  $df = 1$ ,  $P = 0.1$ ). Similarly, there was no significant difference in mortality between the Tas and NSW populations (Yates corrected  $\chi^2 = 2.3$ ,  $df = 1$ ,  $P = 0.13$ ).

From egg to adult, the highest level of mortality at 14°C was experienced by NSW *P. persimilis* on a diet of *T. lintearius* (50%). The lowest level of mortality at 14°C was experienced by Tas *P. persimilis* on a diet of *T. urticae* (20%). Similarly at 24°C, the highest level of mortality from egg to adult was also experienced by NSW *P. persimilis* on a diet of *T. lintearius* (25%). The lowest level of mortality at 24°C was experienced by both Tas and NSW *P. persimilis* on a diet of *T. urticae* (10%) (Table 3.1). The majority of deaths for all treatments occurred in the egg, larval and protonymph stages (Table 3.1). The mean mortalities irrespective of population, diet and temperature were 39.5% (egg), 23.7% (larvae) and 34.3 % (protonymph). The deutonymph stage experienced relatively little mortality (2.6%).

**Table 3.1.** Mortality of pre-adult life stages of NSW and Tas populations of *P. persimilis* reared on diets of *T. urticae* and *T. lintearius* at 14°C and 24°C. Data for egg, larvae, protonymph and deutonymph are the number of deaths that occurred in each lifestage, followed by the number of live *P. persimilis* entering each stage (in parentheses).

		<i>T. urticae</i>		<i>T. lintearius</i>	
Life Stage		Tas	NSW	Tas	NSW
14°C	Egg	2 (20)	4 (20)	0 (20)	4 (20)
	Larvae	2 (18)	0 (16)	2 (20)	2 (16)
	Protonymph	0 (16)	2 (16)	3 (18)	4 (14)
	Deutonymph	0 (16)	1 (14)	0 (15)	0 (10)
	<b>Egg to adult mortality</b>	<b>20 %</b>	<b>35 %</b>	<b>25 %</b>	<b>50 %</b>
24°C	Egg	1 (20)	2 (20)	2 (20)	0 (20)
	Larvae	1 (19)	0 (18)	0 (18)	2 (20)
	Protonymph	0 (18)	0 (18)	1 (18)	3 (18)
	Deutonymph	0 (18)	0 (18)	0 (17)	0 (15)
	<b>Egg to adult mortality</b>	<b>10 %</b>	<b>10 %</b>	<b>15 %</b>	<b>25 %</b>

### 3.3.2 Determination of development times

The mean development time of *P. persimilis* from egg to adult was 17.8 days at 14°C (Table 3.2 a). At this temperature, diet had no significant effect on the development time of *P. persimilis* from protonymph to adult ( $F_{1,50} = 0.12$ ,  $P = 0.74$ ). Similarly, there was no significant difference between the development time from protonymph to adult of Tas and NSW *P. persimilis* populations ( $F_{1,50} = 1.0$ ,  $P = 0.32$ ).

At 24°C, the mean development time of *P. persimilis* from egg to adult was 5.8 days (Table 3.2 b). There was no significant difference between the development time from protonymph to adult of the Tas and NSW *P. persimilis* populations at this temperature ( $F_{1,64} = 0.90$ ,  $P = 0.35$ ). However, in contrast to the lower temperature of 14°C, there was a significant difference due to diet in the development time of *P. persimilis* in the



**Table 3.2.** Mean development time (days  $\pm$  SE) for pre-adult life stages of NSW and Tas populations of *P. persimilis* reared on diets of *T. urticae* (two-spotted mite) and *T. lintearius* (gorse spider mite) at **a)** 14°C and **b)** 24°C. The numbers in parentheses represent the number of individuals comprising the mean.

**a) 14°C**

<i>P. persimilis</i> life stage	<i>T. urticae</i>		<i>T. lintearius</i>	
	Tas	NSW	Tas	NSW
Egg	7.8 $\pm$ 0.07 (18)	7.6 $\pm$ 0.10 (16)	8.0 $\pm$ 0.09 (20)	8.0 $\pm$ 0.12 (16)
Larva	2.2 $\pm$ 0.12 (16)	2.5 $\pm$ 0.12 (16)	2.1 $\pm$ 0.09 (18)	2.4 $\pm$ 0.13 (14)
Protonymph	3.6 $\pm$ 0.16 (16)	3.9 $\pm$ 0.17 (14)	3.9 $\pm$ 0.17 (15)	3.9 $\pm$ 0.16 (10)
Deutonymph	3.9 $\pm$ 0.14 (16)	3.9 $\pm$ 0.15 (13)	3.8 $\pm$ 0.14 (15)	3.8 $\pm$ 0.08 (10)
<b>Egg to adult</b>	<b>17.5 <math>\pm</math> 1.4 (16)</b>	<b>17.9 <math>\pm</math> 1.2 (13)</b>	<b>17.7 <math>\pm</math> 1.1 (15)</b>	<b>18.1 <math>\pm</math> 1.3 (10)</b>

**b) 24°C**

<i>P. persimilis</i> life stage	<i>T. urticae</i>		<i>T. lintearius</i>	
	Tas	NSW	Tas	NSW
Egg	2.5 $\pm$ 0.05 (19)	2.5 $\pm$ 0.06 (18)	2.3 $\pm$ 0.10 (18)	2.4 $\pm$ 0.06 (20)
Larva	0.6 $\pm$ 0.05 (18)	0.6 $\pm$ 0.05 (18)	0.7 $\pm$ 0.06 (18)	0.6 $\pm$ 0.05 (18)
Protonymph	1.4 $\pm$ 0.08 (18)	1.5 $\pm$ 0.04 (18)	1.4 $\pm$ 0.08 (17)	1.4 $\pm$ 0.07 (15)
Deutonymph	1.6 $\pm$ 0.07 a <sup>1</sup> (18)	1.4 $\pm$ 0.08 ab (18)	1.3 $\pm$ 0.07 b (17)	1.2 $\pm$ 0.08 b (15)
<b>Egg to adult</b>	<b>6.1 <math>\pm</math> 0.6 a<sup>1</sup> (18)</b>	<b>6.0 <math>\pm</math> 0.6 a (18)</b>	<b>5.7 <math>\pm</math> 0.6 b (17)</b>	<b>5.6 <math>\pm</math> 0.6 b (15)</b>

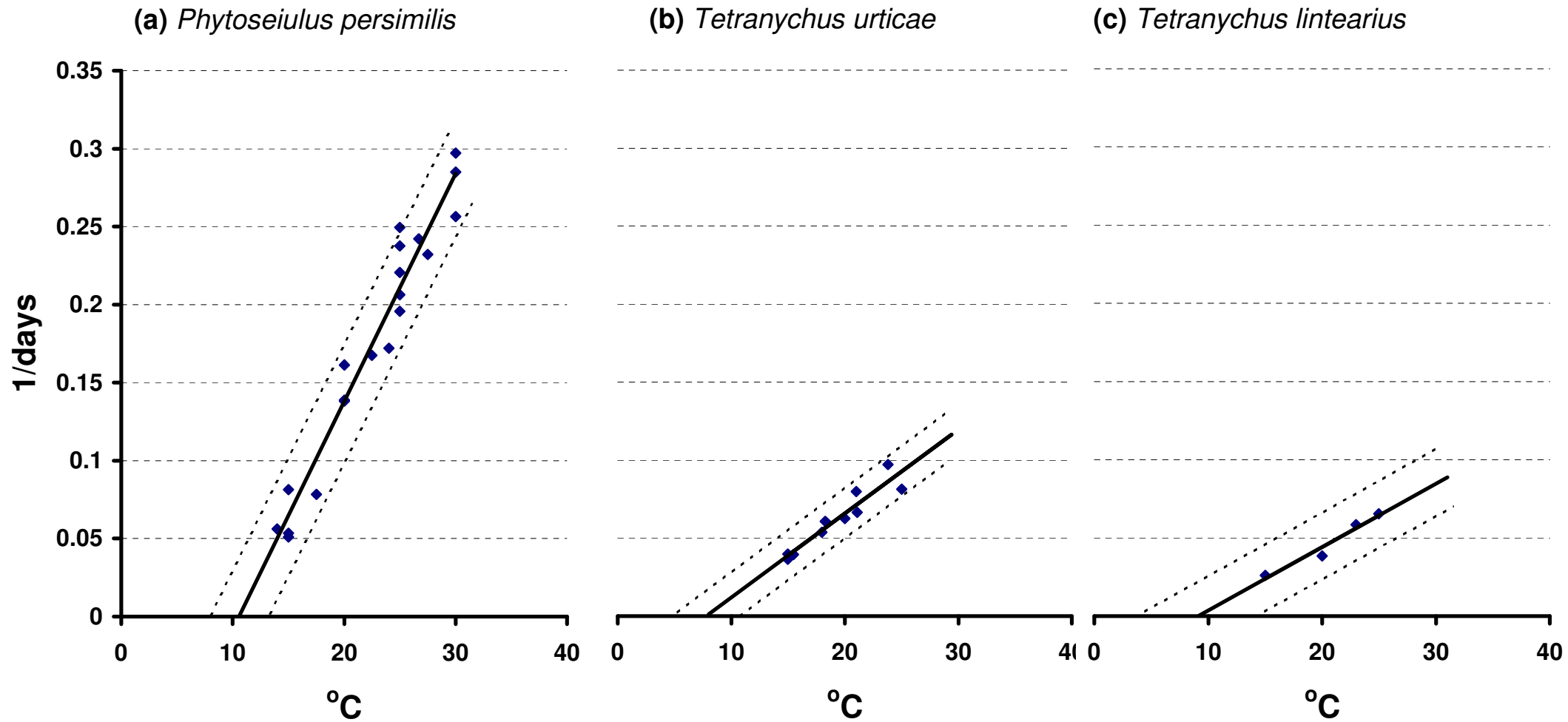
<sup>1</sup>Means with different letters for each lifestage are significantly different (P < 0.05; Fishers LSD).

feeding stages from protonymph to adult ( $F_{1,64} = 7.96$ ,  $P = 0.006$ ). At 24°C, both Tas and NSW *P. persimilis* completed their development from egg to adult almost half a day earlier on a diet of *T. lintearius* (5.6 days) compared to a diet of *T. urticae* (6.0 days) (Table 3.2 b).

When each lifestage at 24°C was analysed independently, there were no significant differences in the development times between Tas and NSW *P. persimilis* on each diet for eggs, larvae or protonymphs (egg:  $F_{3,71} = 1.97$ ,  $P = 0.13$ ; larvae:  $F_{3,68} = 0.39$ ,  $P = 0.76$ ; protonymph:  $F_{3,64} = 0.55$ ,  $P = 0.65$ ). However, there was a significant difference in the development of *P. persimilis* of the deutonymph stage ( $F_{3,64} = 3.67$ ,  $P = 0.017$ ). The Tas *P. persimilis* fed on a diet of *T. urticae* had a significantly longer development time in the deutonymph stage compared to either population on a diet of *T. lintearius*. However, the NSW *P. persimilis* fed on a diet of *T. urticae* did not have a significantly different development time in the deutonymph stage to any of the other combinations (Table 3.2 b).

### 3.3.3 Comparison of development rates and generation times of *P. persimilis*, *Tetranychus urticae* and *Tetranychus lintearius*.

Plots of the development rates (1/days) are provided for *P. persimilis* (Fig. 3.1 a), *T. urticae* (Fig 3.1 b) and *T. lintearius* (Fig. 3.1 c). From egg to adult, *P. persimilis* had the most rapid development rate and the lowest number of day degrees (68.5 DD) to complete development (Table 3.3, Fig. 3.1). For *T. urticae*, a slower development rate resulted in the number of day degrees required to complete development being more than double that of *P. persimilis* (185.2 DD). *T. lintearius* had the slowest development rate with the number of day degrees (243.9 DD) required to complete development 3.5 times that of *P. persimilis*.



**Figure 3.1.** Development rates (1/days) of **a)** *Phytoseiulus persimilis* **b)** *Tetranychus urticae* and **c)** *Tetranychus lintearius*. Broken lines display 95% confidence interval.

**Table 3.3.** Generation times (days from egg-adult), predator: prey generation time ratios (GTR), day degrees (DD) required to complete development and lower development thresholds at 15°C and 25°C for *Phytoseiulus persimilis* (predator), *Tetranychus urticae* (prey) and *T. lintearius* (prey).

	<i>Phytoseiulus persimilis</i>	<i>Tetranychus urticae</i>	<i>Tetranychus lintearius</i>
Equation	$y=0.0146x-0.1549$	$y=0.0054x-0.0419$	$y=0.0041x-0.037$
R <sup>2</sup>	0.9491	0.8929	0.9557
Days (± SE) from egg-adult at 15°C <sup>1</sup>	15.6 ± 4.92	25.6 ± 1.17	40.8 ± 1.08
Predator: prey GTR at 15°C <sup>3</sup>	n/a	0.61	0.38
Days (± SE) from egg-adult at 25°C <sup>2</sup>	4.8 ± 1.55	10.7 ± 1.56	15.3 ± 0.80
Predator: prey GTR at 25°C <sup>3</sup>	n/a	0.45	0.31
DD <sup>4</sup> to complete development	68.5 ± 3.75	185.2 ± 22.6	243.9 ± 36.7
Lower development threshold	10.6 ± 1.2oC	7.8 ± 1.3oC	9.0 ± 1.2oC

<sup>1</sup>Calculated by solving  $y$  on equation when  $x = 15$

<sup>2</sup>Calculated by solving  $y$  on equation when  $x = 25$

<sup>3</sup>Calculated by dividing prey (either *T. urticae* or *T. lintearius*) generation time by predator (*P. persimilis*) generation time.

<sup>4</sup>Day degrees (± SE) above lower development threshold.

The lower development threshold for *P. persimilis* (10.6°C) was higher than either of its prey species. The lower development threshold for *T. lintearius* (9°C) was higher than that for *T. urticae* (7.8°C) (Table 3.3). However, when the 95% confidence intervals are compared there is no distinguishable difference between the lower development thresholds of the three species (Fig. 3.1).

The predator: prey generation time ratios were less than one for both prey species at both 15°C and 25°C and consistently higher between *P. persimilis* and *T. urticae* than

between *P. persimilis* and *T. lintearius*. At 15°C, *P. persimilis* will complete its development in almost half the time of *T. urticae* and less than half the time of *T. lintearius*. At 25°C, *P. persimilis* will complete its development in less than half the time of *T. urticae* and less than a third of the time of *T. lintearius*. (Table 3.3).

### 3.4 Discussion

The performance of Tas and NSW populations of *P. persimilis* was similar on both diets tested at 14°C. At 24°C, performance varied as there was a significantly higher mortality rate and a significantly shorter development time on a diet of *T. lintearius* compared to a diet of *T. urticae*. However, these results confirm that *T. lintearius* is a suitable prey species for *P. persimilis*. Even with a higher mortality on a diet of *T. lintearius*, the ability of *P. persimilis* to build up in large numbers under field conditions and suppress *T. lintearius* populations has been observed (Ireson *et al.*, 2003; Pratt *et al.*, 2003).

Irrespective of diet, the mortality and development of both Tas and NSW *P. persimilis* was the same at both temperatures, indicating that the performance of the Tasmanian field population of *P. persimilis* is comparable to the commercially available population from NSW. As no difference in physiological traits could be detected, there is no evidence that the two populations are behaving as different strains according to Clarke and Walters (1995) definition.

Predators with a smaller predator: prey generation time ratio ( $<1$ ) may be more effective biological control agents of a particular pest arthropod than predators with a larger predator: prey generation time ratio ( $>1$ ) (Dixon *et al.*, 1997). In this study, we are applying the generation time ratio hypothesis to a predator of a weed biological control agent rather than a predatory biological control agent of a pest arthropod. However as it is a typical predator: prey relationship, the generation time ratio hypothesis should still apply equally to both situations.

It has been shown in previous studies that *P. persimilis* has a significant impact on the abundance of *T. urticae* and is indeed a very effective biological control agent of this

species (McMurtry and Croft, 1997). Compared to *T. urticae*, *T. lintearius* has a relatively longer generation time and therefore the predator: prey generation time ratio is even smaller. Therefore, in accordance with the generation time ratio hypothesis (Dixon, *et al.*, 1997), it is likely that the impact of *P. persimilis* on *T. lintearius* populations will be as great or even greater than its impact on *T. urticae* populations.

In the Tasmanian midlands, it was demonstrated that *T. lintearius* can have a significant impact on gorse growth and therefore has the potential to be a useful biological control agent (see Chapter 2). This reduction in growth was measured over 2.5 years from the initial establishment of *T. lintearius* at the site. Two predators, the predatory ladybird beetle, *Stethorus* sp. and *P. persimilis*, were first recorded at the site eight and ten months respectively after the establishment of *T. lintearius*. No quantitative data on the impact of the predators on *T. lintearius* was obtained in this study. It is therefore unknown if the impact of *T. lintearius* would have increased significantly in the absence of the predation. However, it is possible that the maximum level of control that predation could have exerted would not have been evident until after the trial was concluded as predator population densities may not have reached maximum levels until then. Surveys by Ireson *et al.* (2003) concluded that *P. persimilis* is widespread in *T. lintearius* populations throughout much of Tasmania and have associated this predator with the destruction of entire colonies of *T. lintearius*. This association is supported by predator exclusion studies in the USA by Pratt *et al.* (2003), who showed that predation by *P. persimilis* can significantly reduce the size of *T. lintearius* colonies.

Predation of *T. urticae* by *P. persimilis* can result in localised extinction in an enclosed, protected environment such as a glasshouse and is therefore a very effective

biological control agent of *T. urticae* in this situation. However, in a natural environment prey dispersal, and asynchrony in predator and prey populations will result in a patchy equilibrium, with alternating prey overpopulation and localised extinctions occurring (Pels and Sabelis, 1999).

Presumably, similar dynamics between *T. lintearius* and *P. persimilis* will occur and asynchronous predator and prey populations will develop throughout gorse populations. This may result in a low occurrence of the higher damage levels measured by Davies (see Chapter 2) with low levels of patchy damage to gorse becoming the norm once predator and prey populations stabilise. Evidence that this is occurring in Tasmania is provided by the widespread but localised damage that is now being observed at sites around the state. This contrasts with the widespread and highly damaging outbreaks of *T. lintearius* that were observed during the first two or three years following its initial release into the state (J. E. Ireson, pers. comm.).

In conclusion, this study provides further evidence that predation by *P. persimilis* is having a deleterious effect on *T. lintearius* populations. It is therefore likely that the level and frequency of damage to gorse by *T. lintearius* will be lower than would otherwise have been recorded in the absence of *P. persimilis*.



## Chapter 4. The phenology and impact of the gorse seed weevil, *Exapion ulicis*, on gorse, *Ulex europaeus*.

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### Abstract

The patterns of gorse pod production, the phenology of *Exapion ulicis* and its impact on gorse seed production were assessed in a field study conducted at two sites over 20 months in Tasmania, Australia. The production of gorse pods varied considerably between the two sites and as a consequence, the impact of *E. ulicis* on mature gorse seed production varied between the sites. At Stonehenge, a site in Tasmania's eastern midlands, green pods were produced in spring and summer and black pods were produced in late spring, summer and early autumn, which was well synchronised with *E. ulicis* seed feeding activity (spring to early autumn). At Lymington, a coastal site in southern Tasmania, both green and black pods were produced almost all year, which was not well synchronised with *E. ulicis* seed feeding activity (spring to early autumn). The percentage of mature seed in black pods that were damaged by *E. ulicis* during the whole 20 month sampling period was 2.7 times higher at Stonehenge (45.5%) than at Lymington (16.7%). On an annual basis, damage to mature seed at Stonehenge was 34.4% (2001/2002) and 55.4% (2002/2003) and at Lymington was 18.1% (2001/2002) and 12.4% (2002/2003). A New Zealand modelling study (Rees and Hill, 2001) concluded that in excess of 90% of seed would need to be destroyed to have an impact on gorse populations. Therefore, the reduction in seed production due to *E. ulicis* was not enough to have an impact on gorse populations at either site in this study. However, it is possible that *E. ulicis* and an additional introduced seed-feeding agent could together damage enough seed to have an impact on gorse populations at some sites in Tasmania.

## 4.1 Introduction

Gorse, *Ulex europaeus* L. (Fabaceae), is a perennial, spiny shrub which can live up to 30 years of age and grow to more than three metres in height and diameter. Gorse forms dense, impenetrable thickets that compete strongly with pasture and other desirable plant species (Richardson and Hill, 1998) and is a Weed of National Significance (Thorp, 1999). Gorse flowers are primarily produced in spring but the production of flowers outside this period, particularly in autumn and winter, is common (Parsons and Cuthbertson, 2001). Following flowering, large numbers of seed are produced in small pods and can remain viable in soil in excess of 25 years (Moss, 1959). In a study conducted near Auckland, New Zealand (Cowley, 1983), the number of seeds per gorse pod averaged 3.2 (range 1-9).

As part of an integrated management strategy, a guild of host-specific biological control agents are currently under investigation in Australia (Ireson *et al.*, 2004). The gorse seed weevil, *Exapion ulicis* Forster, (Coleoptera: Brentidae) was the first agent to be introduced into Australia for gorse control. Originally from Europe, this species was introduced to Tasmania in 1939 from a population that had previously established in New Zealand (Evans, 1943). *E. ulicis* is now widely distributed throughout Tasmania and Victoria and has also been recorded in South Australia and New South Wales (Ireson *et al.*, 2006).

In New Zealand, adult *E. ulicis* are present outside pods feeding on gorse foliage and flowers for the majority of the year. In spring, adult females chew a hole in young green gorse pods between 10 and 35 days old then oviposit a clutch of approximately 7 eggs (range 1-19) inside the pods (Cowley, 1983). The oviposition hole heals over, eggs hatch and three larval instars develop within the ripening pods, feeding on the

developing seed within. Pods turn dark as they ripen, pupation takes place inside the pods and adult *E. ulicis* emerge when the seed ripens and pods dehisce. As the larvae of this species feed and develop within gorse pods they destroy a proportion of the seed (Hill *et al.*, 1991).

Miller (1947) predicted that there was a good chance of controlling gorse using *E. ulicis* alone in New Zealand, however this didn't occur due to a lack of synchrony between *E. ulicis* reproductive activity and gorse pod production (Hill *et al.* 1991). In New Zealand, *E. ulicis* is univoltine as in its native range in Europe. However in New Zealand, gorse often produces two crops of seed per year and *E. ulicis* only attacks the second crop, which is produced between July and December (Cowley, 1983). In the same study, *E. ulicis* attack effectively reduced the viable spring seeds from around three per pod to one per four attacked pods in attacked pods in spring, however, only 36% of the pods produced annually were attacked. In an insecticide exclusion study also conducted in New Zealand, Hill *et al.* (1996) found that *E. ulicis* reduced seed production by approximately 45%.

The aim of this study was to determine the timing of gorse pod production and *E. ulicis* attack at two sites in south-east Tasmania. Furthermore, it aimed to quantify the seed feeding activities of *E. ulicis* to determine its impact on gorse seed production.

## 4.2 Materials and Methods

This study involved regular sampling of gorse pods at two to four week intervals at two sites in south-eastern Tasmania between winter 2001 and autumn 2003. Two sites were selected based on previously observed differences in the timing of pod production.

### 4.2.1 Study sites

The Stonehenge site was located on a sheep grazing property near Stonehenge, Tasmania (42° 23' S, 147° 39' E). This site was situated adjacent to the Little Swanport river, approximately 30 km from the east coast of Tasmania and 300 m above sea level. Gorse was growing amongst semi-improved pasture consisting of a mix of native grasses and introduced grass and clover species. Gorse bushes at this site were approximately five years of age and between one and two metres in height and diameter. Stonehenge is in an inland region at 300 m above sea level and the annual temperature of the nearest weather station (Oatlands) ranges between a daily mean minimum temperature of 5°C and a daily mean maximum temperature of 15.4°C (Bureau of Meteorology, 2005).

The Lymington site was located on a hobby farm near Lymington, Tasmania (43° 11' S, 147° 01' E). This site was approximately 1 km from the coast near Port Cygnet and 20m above sea level. The gorse infestation was on a hillside with a north-easterly aspect growing amongst introduced pasture species and adjacent to native tree species (eg. *Acacia dealbata* and *Eucalyptus* spp.). Gorse bushes at this site were approximately seven years of age and between one and three metres in height and diameter. Lymington is close to the coast at 20 m above sea level and the annual temperature of the nearest weather station (Geeveston) is slightly warmer than

Stonehenge, ranging between a daily mean minimum temperature of 6.7°C and a daily mean maximum temperature of 17.4°C (Bureau of Meteorology, 2005).

#### *4.2.2 Sampling procedure*

At each site, 30 easily accessible gorse bushes were randomly chosen and tagged. The same bushes were used throughout the sampling period. Sampling was conducted for 19 months between August 2001 and March 2003 at Stonehenge. At Lymington, sampling was initiated slightly earlier and was conducted for 20 months between July 2001 and March 2003.

On each sampling date, conducted at intervals between two and four weeks, the 30 bushes were inspected for the presence or absence of pods at two stages of development. The younger pods, in which the tip of the pod had appeared through the sepals and the pod was more than 50% green in colour were designated as 'green' pods. The more mature pods that were greater than 50% black in colour were designated as 'black' pods. On each plant, if more than 10 of the green or of the black pods could be found in a 30 second period, a subsample was taken. This consisted of 10 pods per plant of both or either stage that were haphazardly collected and taken back to the laboratory for further assessment. At the Stonehenge site, the total number of flowers and pods on some plants were so low that if 10 of each were taken on each sampling date, there would not have been enough for subsequent sampling dates. It was decided that for these particular plants (five plants in total) that only five pods of each stage would be collected at each sampling date when pods were present.

Both green and black pods were assessed for the number of gorse seeds and number of the different *E. ulicis* stages within the pods. Pods were split along the suture with a scalpel to reveal the seed. The numbers of eggs, larvae, pupae and adult *E. ulicis* were

recorded together with the number of damaged and undamaged seeds. This step involved the use of a dissecting microscope at  $10\times$  magnification and careful movement of the seed to reveal hidden *E. ulicis* stages.

In some cases, *E. ulicis* had damaged seed within pods to such an extent that seed counts could not be made. In these cases, the mean number of seeds in green or black pods at each site was used as an estimate of the number of seed within a pod where extensive *E. ulicis* damage had occurred.

The percentage of pods that were infested and percentage of seed in black pods damaged by *E. ulicis* were calculated for each site over the entire sampling periods and on an annual basis. The first annual period was August 2001 to July 2002 and the second period was April 2002 to March 2003. As the sampling was conducted over 19 and 20 months for Stonehenge and Lymington respectively, there was overlap between the two annual periods.

#### 4.2.3 Data analysis

Statistical tests were performed using SYSTAT 10th edition. To determine if there was a difference in pod production between the two sites, the proportion of sample dates in the study that each plant had pods was initially calculated. This data was arcsine square root transformed then subjected to an analysis of variance. To determine if there was a difference in infestation levels of pods by *E. ulicis* between the two sites, the proportion of green and black pods produced that were infested by *E. ulicis* on each plant was calculated. This data was arcsine square root transformed then subjected to an analysis of variance.

To determine what *E. ulicis* life stage was the key factor contributing most to total mortality among generations, stage specific life tables were constructed and key factor (k values) analysis undertaken (see Kidd and Jervis, 1996). The mortality suffered by each *E. ulicis* life stage was calculated from the mean number of each stage found per pod at both sites (Stonehenge and Lymington) over both seasons (2001/2002 and 2002/2003). Multivariate regression procedures were used to explore the relationship between the number of seeds in a pod and the number of eggs laid in a pod with site and season and all interactions included in the full model and stepwise elimination procedures then used to produce the most parsimonious model.

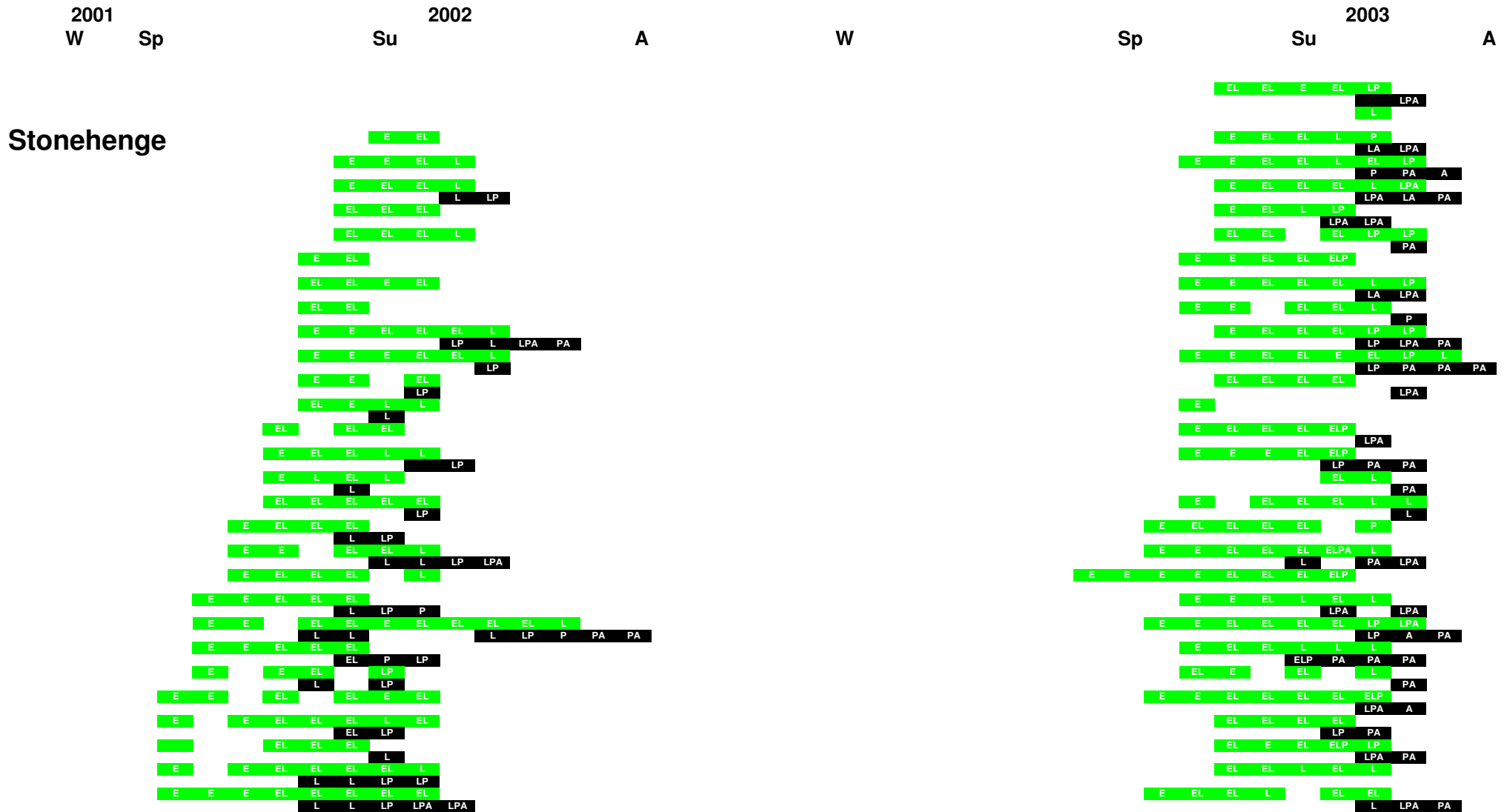
### 4.3 Results

#### 4.3.1 Pod production

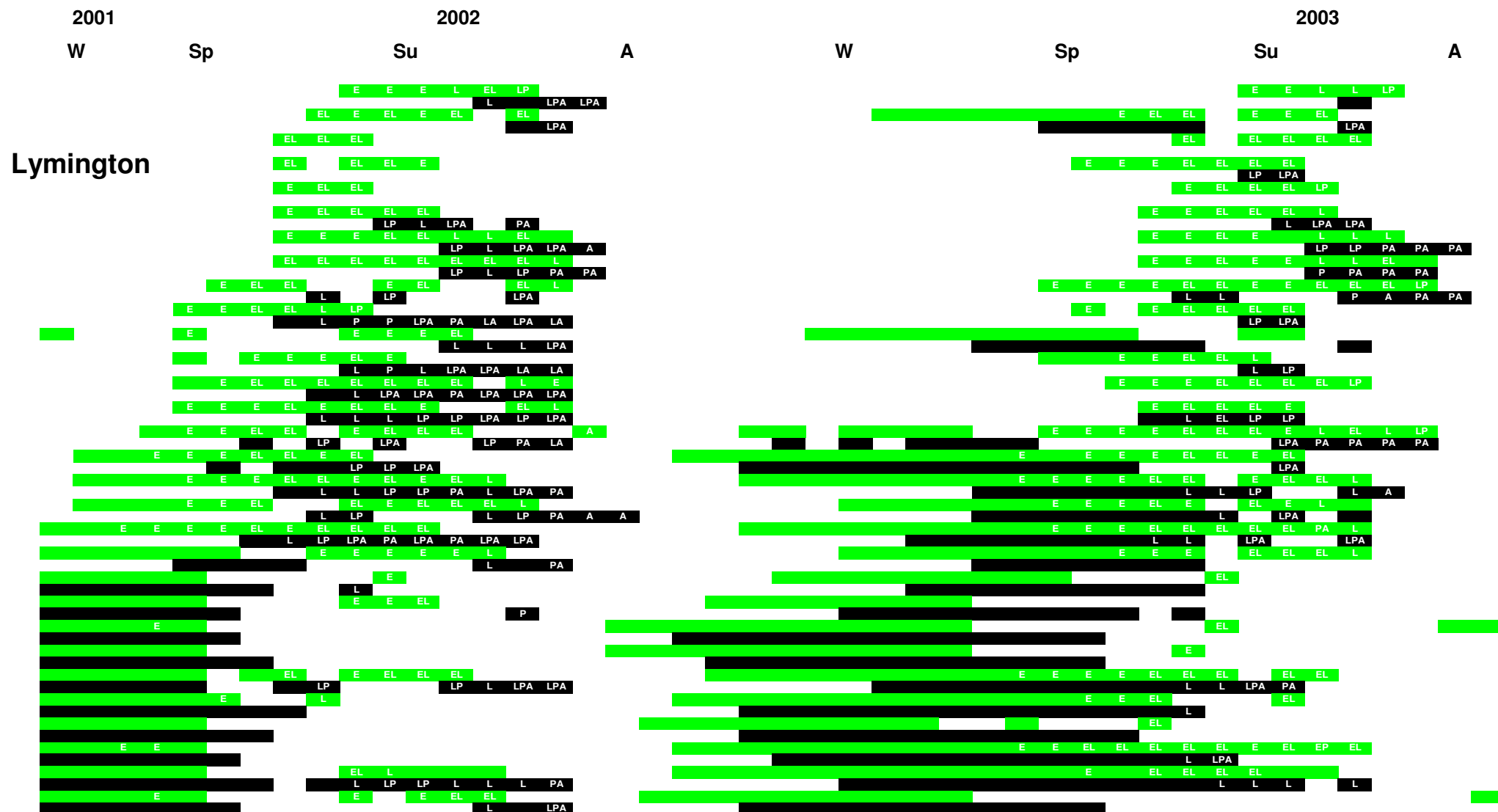
Stonehenge had pods over a significantly shorter time than Lymington with a significant difference between the two sites in the proportion of sample dates that each plant had pods (Green pods:  $F_{1,58} = 44.4$ ,  $P < 0.01$ ; Black pods:  $F_{1,58} = 45.9$ ,  $P < 0.01$ ). Out of a total of 43 sample dates over 19 months at Stonehenge, green pods were present on gorse bushes at a mean of  $22.5 \pm 1.4\%$  of sample dates (range 2.3% to 41.9%,  $n = 30$ ). Black pods were present on gorse bushes a mean of  $7.6 \pm 1.1\%$  of sample dates (range 0% to 23.3%,  $n = 30$ ). At Stonehenge, green pods were present on bushes during spring, summer and early autumn (from 19/9/2001 to 14/2/2002 and 10/9/02 to 15/1/03) (Figs. 4.1a, 4.2a). Black pods were present on bushes slightly later during late spring, summer and early autumn in 2001/2002 (from 7/11/2001 to 13/3/2002) and summer only in 2002/2003 (from 3/12/2002 to 12/2/2003) (Figs. 4.1a, 4.2b).

By contrast, at Lymington, green pods were present on twice as many and black pods were present on more than three times as many sample dates than Stonehenge. Out of a total of 44 sample dates over 20 months at Lymington, green pods were present on a mean of  $44.2 \pm 2.9\%$  of sample dates (range 18.2% to 70.5%,  $n = 30$ ) and black pods were present on a mean of  $31.6 \pm 3.0\%$  of sample dates (range 0% to 68.2%,  $n = 30$ ). At Lymington, green pods were present on bushes all year round with no sample dates without green pods found on at least some bushes (Figs 4.1b and 4.2c). Similarly, black pods were also present nearly all year except for a short window in Autumn (March) each year (Figs 4.1b and 4.2d).



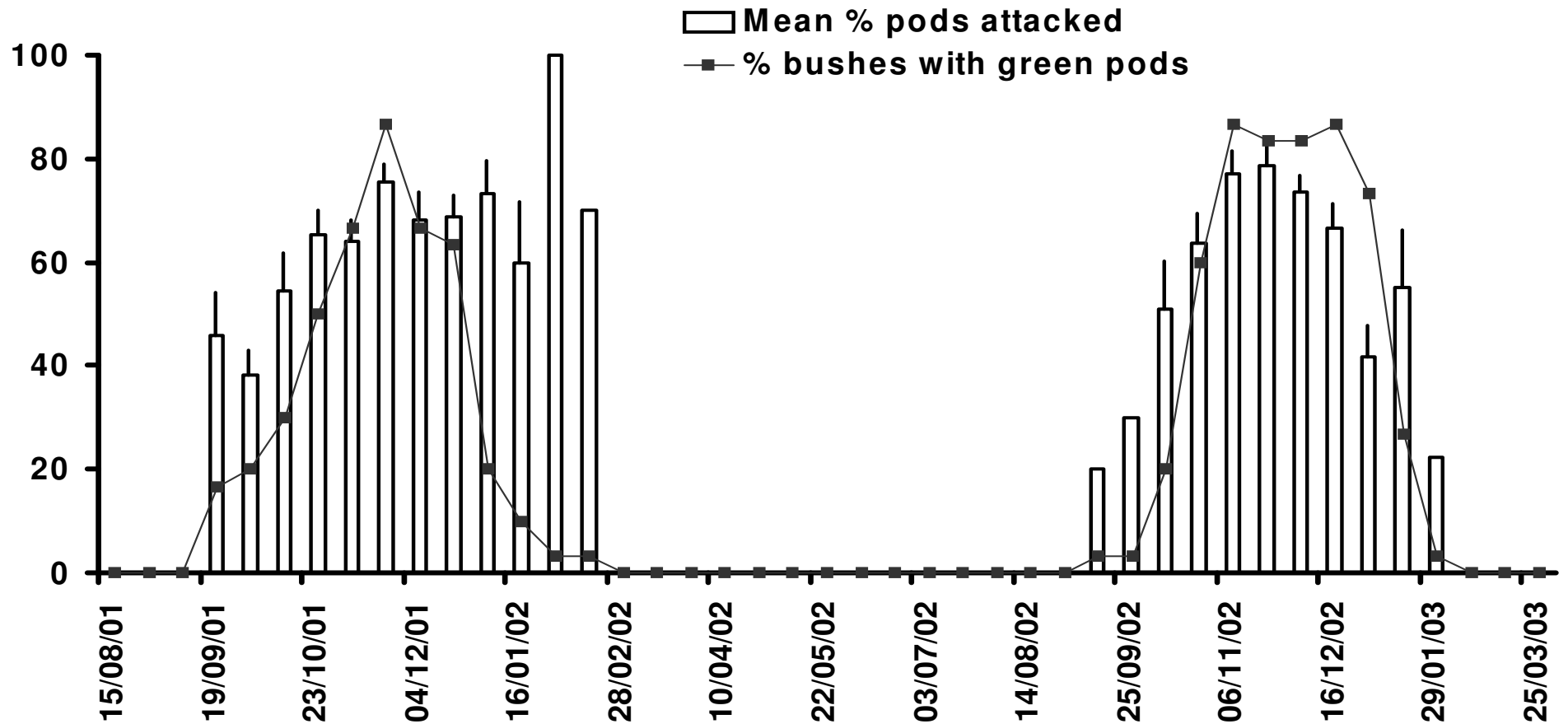


**Figure 4.1(a).** Phenology of green (■) and black (■) pod production of individual gorse plants (total of 30 plants at each site) and *E. ulicis* stages within gorse pods (E = eggs, L = larvae, P = pupae and A = adults) between winter 2001 and autumn 2003 (W = winter, Sp = spring, Su = Summer, A = autumn) at Stonehenge. Each vertically adjacent green and black bar represents a single plant.



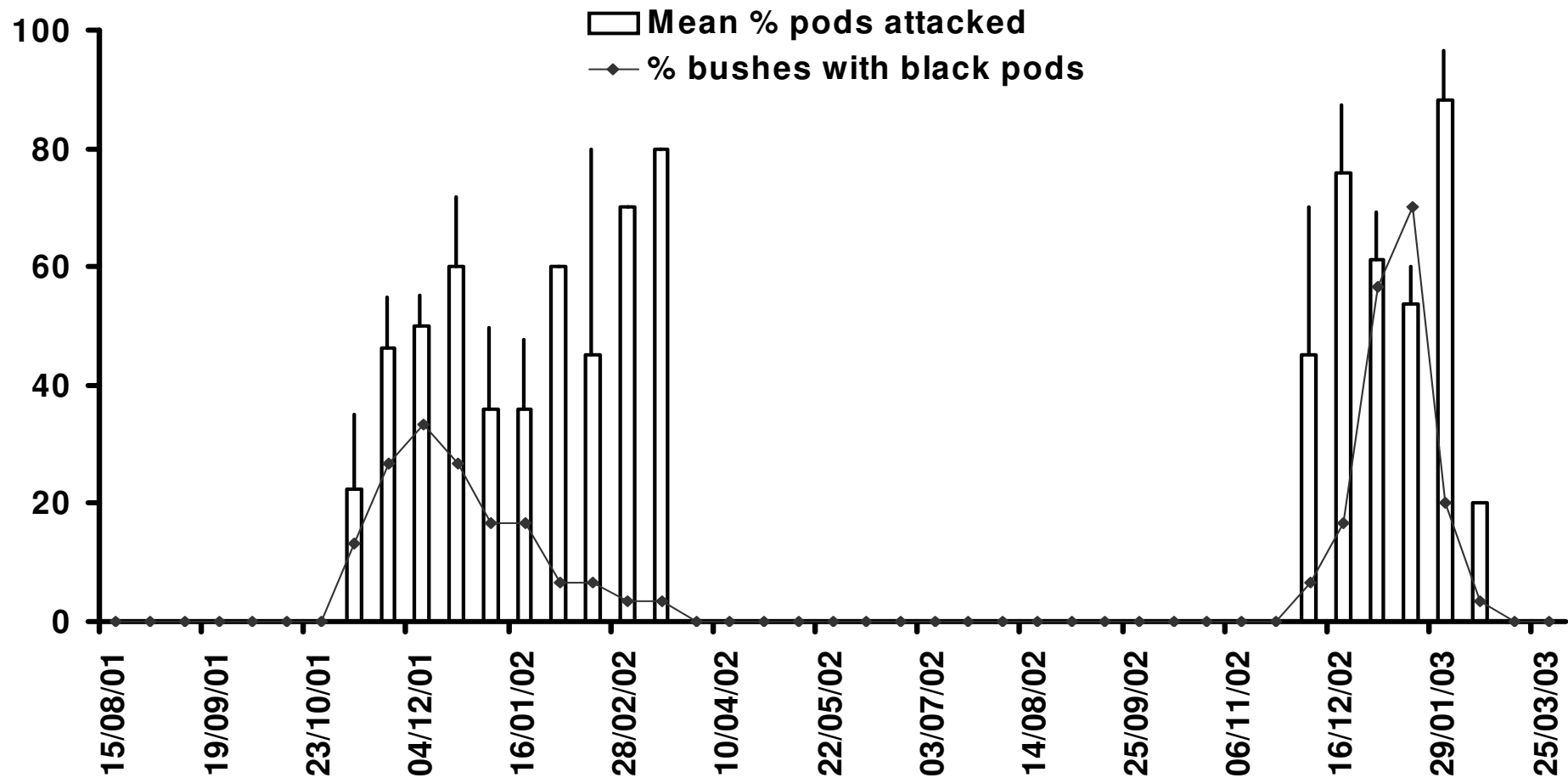
**Figure 4.1(b).** Phenology of green (■) and black (■) pod production of individual gorse plants (total of 30 plants at each site) and *E. ulicis* stages within gorse pods (E = eggs, L = larvae, P = pupae and A = adults) between winter 2001 and autumn 2003 (W = winter, Sp = spring, Su = Summer, A = autumn) at Lymington. Each vertically adjacent green and black bar represents a single plant.

## Stonehenge – green pods



**Figure 4.2(a).** Mean ( $\pm$  SE) percentage of green pods attacked by *Exapion ulicis* and percentage of bushes (n=30) with green pods at Stonehenge.

# Stonehenge – black pods



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**Figure 4.2(b).** Mean ( $\pm$  SE) percentage of black pods attacked by *Exapion ulicis* and percentage of bushes (n=30) with black pods at Stonehenge.

# Lymington – green pods

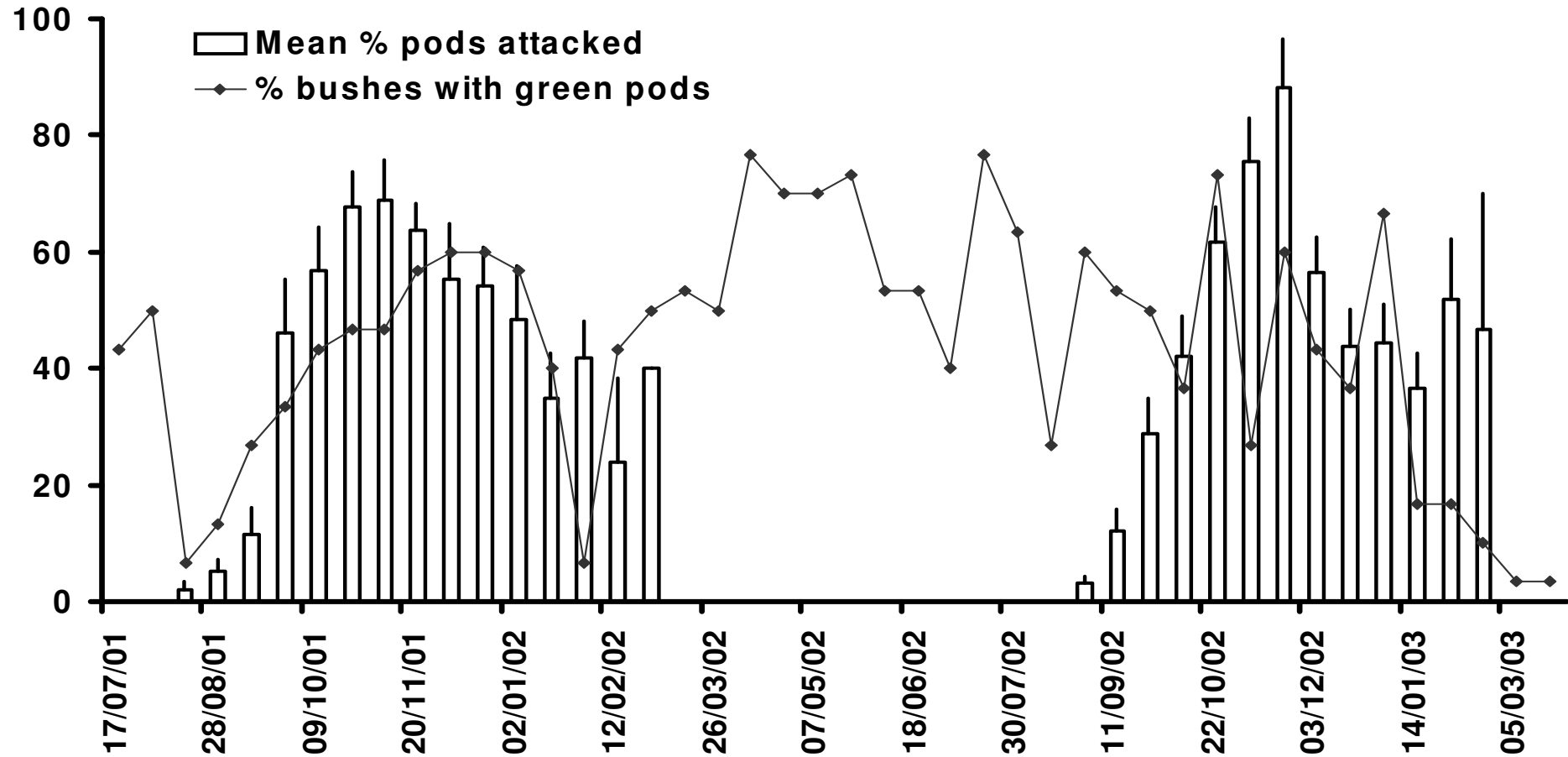
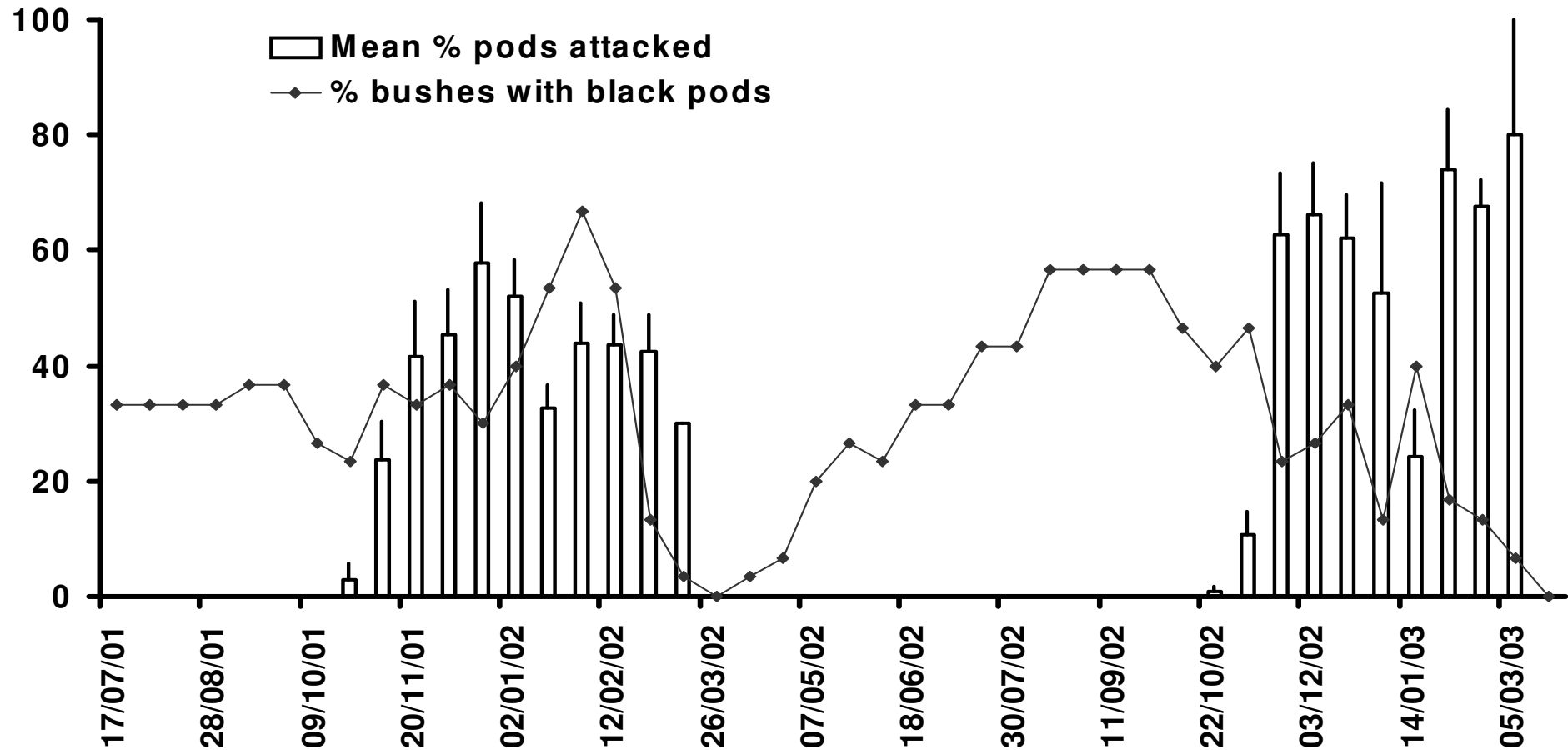


Figure 4.2(c). Mean ( $\pm$  SE) percentage of green pods attacked by *Exapion ulicis* and percentage of bushes (n=30) with green pods at Lymington.

# Lymington – black pods



58 **Figure 4.2(d).** Mean ( $\pm$  SE) percentage of black pods attacked by *Exapion ulicis* and percentage of bushes (n=30) with black pods at Lymington.

#### 4.3.2 Phenology of *E. ulicis*

At Stonehenge, *E. ulicis* eggs were found as soon as green pods first appeared in both seasons. Eggs were present in pods in spring and summer (19/9/2001 to 30/1/2002 and 10/9/2002 to 3/1/2003) (Table 4.1a, Fig. 4.1a), with the maximum number of eggs found on a sampling date occurring in mid to late spring (21/11/2001 and 6/11/2002) (Table 4.1a). Larvae were also present in pods in spring and summer, although slightly later than eggs (23/10/2001 to 14/2/2002 and 23/10/2002 to 29/1/2003), with the maximum number of larvae found on a sampling date occurring in late spring to early summer (18/12/2001 and 20/11/02) (Table 4.1a). Pupae were present in pods in summer and early autumn (4/12/2001 to 13/3/2002 and 3/12/2002 to 12/2/2003), with the maximum number of pupae found on a sampling date occurring in summer (4/12/2001 and 15/1/2003) (Table 4.1a). Finally, adults were also present in pods at Stonehenge in summer and early autumn (18/12/2001 to 13/3/2002 and 16/12/2002 to 12/2/2003), with the maximum number of adults found on a sampling date occurring in summer (14/2/2002 and 15/1/2003) (Table 4.1a). Across all sampling dates at Stonehenge there was a mean per pod of  $9.0 \pm 0.20$  eggs (range 1-60,  $n = 1093$  pods),  $5.8 \pm 0.11$  larvae (range 1-21,  $n = 1030$  pods),  $4.7 \pm 0.18$  pupae (range 1-21,  $n = 270$  pods) and  $5.9 \pm 0.18$  adults (range 1-14,  $n = 223$  pods) of *E. ulicis* in infested pods.

At Lymington, *E. ulicis* was recorded in pods earlier than at Stonehenge. For both of the 2001/2002 and 2002/2003 seasons, eggs, larvae, pupae and adults were all first recorded between two and five weeks earlier at Lymington than Stonehenge (Table 4.1a and b). *E. ulicis* eggs were present in pods in late winter, spring and summer (14/8/2001 to 12/2/2002 and 27/8/2002 to 28/1/2003) (Table 4.1b, Fig. 4.1b), with the maximum number of eggs found on a sampling date occurring in mid spring

**Table 4.1(a).** Number of eggs (E), larvae (L), pupae (P) and adults (A) of *E. ulicis* within pods of 30 gorse plants at Stonehenge. Numbers in parentheses indicate number of pods infested by each stage, numbers in bold indicate the maximum number of each stage recorded per season. The total number of damaged (D) and undamaged (U) seed from the total number of green and black pods are also shown.

### Stonehenge

	Date	E	L	P	A	D	U	Pods
W	15/8/01							0
	29/8/01							0
	12/9/01							0
Spring	19/9/01	135 (23)					217	50
	26/9/01	160 (23)					265	60
	10/10/01	395 (49)					357	90
	23/10/01	939 (80)	148 (45)			8	532	150
	7/11/01	1073 (89)	404 (63)			127	687	240
Summer	21/11/01	<b>1162 (118)</b>	540 (90)			146	703	280
	4/12/01	556 (63)	510 (92)	<b>125 (28)</b>		231	542	265
	18/12/01	326 (51)	<b>517 (102)</b>	103 (22)	5 (1)	241	452	240
	3/1/02	38 (6)	254 (50)	35 (8)	8 (1)	114	209	110
	16/1/02	11 (2)	130 (27)	52 (10)	9 (2)	87	208	80
	30/1/02	5 (2)	69 (13)	36 (7)	9 (1)	62	55	30
	14/2/02		40 (7)	12 (2)	<b>37 (7)</b>	44	70	30
	28/2/02			22 (4)	13 (3)	18	11	10
	13/3/02			16 (4)	31 (6)	20	11	10
	27/3/02							0
Autumn	10/4/02							0
	24/4/02							0
	7/5/02							0
	22/5/02							0
	5/6/02							0
	19/6/02							0
Winter	3/7/02							0
	17/7/02							0
	31/7/02							0
	14/8/02							0
	28/8/02							0
Spring	10/9/02	12 (2)					33	10
	25/9/02	19 (3)					40	10
	9/10/02	283 (30)					169	60
	23/10/02	910 (114)	7 (4)				612	180
	6/11/02	<b>1741 (187)</b>	88 (23)			14	850	255
	20/11/02	969 (101)	<b>903 (123)</b>			213	583	245
Summer	3/12/02	635 (85)	829 (127)	1 (1)		298	535	265
	16/12/02	425 (62)	797 (121)	246 (50)	49 (12)	397	599	310
	3/1/03	9 (3)	633 (116)	236 (53)	357 (56)	550	669	385
	15/1/03		136 (33)	<b>344 (71)</b>	<b>520 (85)</b>	441	417	290
	29/1/03		13 (2)	41 (9)	263 (47)	139	46	70
	12/2/03			2 (1)	5 (2)	5	28	10
A	5/3/03							0
	25/3/03							0



**Table 4.1(b).** Number of eggs (E), larvae (L), pupae (P) and adults (A) of *E. ulicis* within pods of 30 gorse plants at Lymington. Numbers in parentheses indicate number of pods infested by each stage, numbers in bold indicate the maximum number of each stage recorded per season. The total number of damaged (D) and undamaged (U) seed from the total number of green and black pods are also shown.

## Lymington

	Date	E	L	P	A	D	U	Pods
W	17/7/01						600	230
	31/7/01						620	250
Spring	14/8/01	10(3)					600	250
	28/8/01	33 (8)					640	260
	12/9/01	134 (24)					802	320
	25/9/01	442 (50)					629	220
	9/10/01	424 (53)	45 (11)			9	519	190
Summer	22/10/01	<b>938 (90)</b>	205 (35)			40	559	230
	7/11/01	556 (55)	469 (76)	57 (10)		155	569	255
	20/11/01	879 (96)	<b>514 (82)</b>	109 (23)	4 (1)	178	765	325
	4/12/01	451 (70)	296 (58)	129 (28)	57 (10)	146	698	297
	19/12/01	566 (76)	248 (50)	142 (30)	130 (16)	160	658	276
Autumn	2/1/02	92 (20)	401 (67)	177 (25)	122 (20)	271	482	248
	15/1/02	10 (5)	373 (68)	35 (8)	54 (11)	167	487	239
	29/1/02	35 (6)	260 (67)	<b>218 (47)</b>	184 (29)	247	535	279
	12/2/02	8 (1)	100 (28)	111 (26)	<b>256 (46)</b>	200	392	210
	26/2/02		2 (1)	7 (2)	101 (20)	56	81	50
Winter	12/3/02				19 (3)	7	91	30
	26/3/02						117	40
	9/4/02						242	90
	23/4/02						283	120
	7/5/02						484	189
Spring	21/5/02						541	219
	4/6/02						495	210
	18/6/02						661	270
	2/7/02						654	279
	16/7/02						701	310
Summer	30/7/02						658	298
	14/8/02						703	289
	27/8/02	13 (4)					749	297
	11/9/02	92 (17)					858	316
	24/9/02	286 (44)	4 (1)				901	328
Autumn	8/10/02	517 (63)	1 (1)				738	294
	22/10/02	816 (99)	84 (21)			22	812	315
	5/11/02	<b>1396 (125)</b>	298 (63)			78	866	348
	19/11/02	1374 (107)	<b>753 (108)</b>	31 (5)	4 (1)	173	423	236
	3/12/02	803 (87)	470 (81)	112 (22)	17 (3)	194	662	295
Winter	16/12/02	561 (70)	452 (65)	<b>228 (37)</b>	71 (15)	191	790	323
	2/1/03	83 (16)	384 (65)	123 (19)	83 (11)	218	411	220
	14/1/03	69 (11)	256 (47)	81 (13)	93 (14)	154	546	240
	28/1/03	10 (3)	107 (20)	83 (17)	<b>193 (29)</b>	140	116	100
	11/2/03		36 (7)	107 (19)	112 (17)	102	71	70
A	5/3/03			3 (2)	91 (16)	38	48	30
	25/3/03						55	20

(approximately 22/10/2001 and 5/11/2002) (Table 4.1b). Larvae were also present in pods in spring and summer, slightly later than eggs (9/10/2001 to 26/2/2002 and 24/9/2002 to 11/2/2003). The maximum number of larvae found on a sampling date was in late spring (20/11/2001 and 19/11/2002) (Table 4.1b). Pupae were present in pods in late spring and summer (7/11/2001 to 26/2/2002 and 19/11/2002 to 5/3/2003), with the maximum number of pupae found on a sampling date occurring in summer (29/1/2002 and 16/12/2003) (Table 4.1b). Finally, adults were also present in pods at Lymington in late spring and summer (20/11/2001 to 12/3/2002 and 19/11/2002 to 5/3/2003). The maximum number of adults found on a sampling date was in summer (12/2/2002 and 28/1/2003) (Table 4.1b, Fig. 4.1b). Across all sampling dates at Lymington there was a mean of  $8.8 \pm 0.20$  eggs (range 1-69,  $n = 1203$  pods),  $5.6 \pm 0.12$  larvae (range 1-29,  $n = 1021$  pods),  $5.3 \pm 0.12$  pupae (range 1-17,  $n = 333$  pods) and  $6.1 \pm 0.18$  adults (range 1-17,  $n = 262$  pods) of *E. ulicis* infested pods.

The population dynamics of *E. ulicis* across both sites indicates that the greatest contributor to overall mortality of generations lay in egg mortality with key factor values between 0.138 and 0.250 for egg mortality. Regression of each life stage key factor against total generation mortality, similarly identify egg mortality as the key factor by virtue of its greatest slope (egg: slope ( $b$ ) = 0.84, larvae  $b = -0.07$ , pupae  $b = 0.27$ ). Apparently driving this egg mortality was the extraordinary number of eggs laid per pod, which ranged up to 60 at Stonehenge and 69 at Lymington. Interestingly, the two sites significantly differed in the mean numbers of seeds per pod ( $t_{1, 2294} = 11.85$ ,  $p < 0.000001$ ), averaging only  $2.8 \pm 0.04$  seeds per pod (range 0-9,  $n = 1203$ ) at Lymington but  $3.6 \pm 0.05$  seeds per pod (range 0-10,  $n = 1093$ ) at Stonehenge.

Across both sites and seasons there was a significant positive relationship between the number of *E. ulicis* eggs laid in a pod and the number of seeds in a pod ( $F_{1,2292} = 8.43$ ,  $P < 0.005$ ), with there further being a significant 2-way interaction between season and location ( $F_{1,2292} = 23.64$ ,  $P < 0.000001$ ) and a significant 3-way interaction between season, location and seed number ( $F_{1,2292} = 12.40$ ,  $P < 0.0005$ ). This egg laying behaviour resulted in an inversely density dependent egg laying pattern with the ratio of eggs laid to seed number in a pod declining with increasing seed number in a pod ( $F_{1,2266} = 758.8$ ,  $P < 0.000001$ ). Furthermore, the proportion of seeds that were eventually damaged in a pod was also inversely density dependent with the percentage of seeds damaged decreasing with increasing seed numbers per pod, especially at Stonehenge ( $F_{1,3308} = 27.8$ ,  $P < 0.000001$ ) and to a lesser extent at Lymington ( $F_{1,9891} = 5.45$ ,  $P = 0.0195$ ).

#### 4.3.3 Impact of *E. ulicis*

A significantly greater proportion of pods on each plant were infested by *E. ulicis* at Stonehenge than at Lymington (green pods:  $F_{1,58} = 38.01$ ,  $P < 0.01$ ; black pods:  $F_{1,53} = 25.81$ ,  $P < 0.01$ ). There was also a significant difference in the proportion of green and black pods on each plant that were infested by *E. ulicis* ( $F_{1,111} = 7.0$ ,  $P < 0.01$ ). At Stonehenge, some level of infestation for green or black pods was recorded on all sampling dates as pods were produced only during periods of *E. ulicis* activity in spring and summer (Fig. 4.2a and b; Table 4.2a). For green pods, infestation levels on individual sample dates ranged between 20% and 100% and black pods ranged between 20% and 88%. At Stonehenge across all sampling dates (August 2001 to March 2003), 64.6 % of all green pods and 54.2 % of all black pods were infested by *E. ulicis* on each of the 30 bushes. On an annual basis at Stonehenge 63.3 % and 65.6

% of all green pods and 45.5 % and 61.2 % of all black pods (Table 4.3) were infested by *E. ulicis* for the annual periods of August 2001 to July 2002 and April 2002 to March 2003 respectively.

In contrast, although the timing of attack was similar at Lymington (albeit slightly earlier), green and black pods were only infested for approximately half of the sampling dates as pods were produced almost all year (Fig. 4.2c and d; Table 4.2b). For green pods, infestation levels on individual sample dates ranged between 0 and 88.1% and black pods ranged between 0 and 80%. Across all sampling dates at Lymington, the levels of infestation by *E. ulicis* were less than half that of Stonehenge for both green and black pods. Across all sampling dates over the 20 months of sampling at Lymington (July 2001 to March 2003), 30.3% of all green pods and 16.7% of all black pods were infested by *E. ulicis* on each of the 30 bushes. On an annual basis 24.7% and 26.8% of all green pods and 19.6% and 17.6% of all black pods (Table 4.3) were infested by *E. ulicis* for the annual periods of August 2001 to July 2002 and April 2002 to March 2003 respectively.

The damage to mature seed within black pods followed a similar pattern to the pod infestation levels. At Stonehenge, there was some level of damage on all sampling dates when black pods were present (Table 4.2a). Damage to seed in black pods occurred from late spring to early autumn (7/11/2001 to 13/3/2002 and 3/12/2002 to 12/2/2003) and ranged between a minimum of 11% and a maximum of 82.5% of all seed. There were no sampling dates where no damage to seed occurred during these periods (Table 4.2a).

In contrast, although damage to black pods occurred at Lymington over a slightly more extended period from late spring to early autumn (22/10/2001 to 12/3/2002 and

22/10/2002 to 5/3/2003) (Table 4.2 b), black pods were produced over a much more extended period (17/7/2001 to 12/3/2002 and 9/4/2002 to 5/3/2003). The damage to all seed produced on each sampling date ranged between a minimum of 0% and a maximum of 70.6% during these periods and there were 21 sampling dates where no damage to seed occurred out of a total of 42 sampling dates when black pods were present on at least some bushes (Table 4.2b).

As a consequence, the percentage of all mature seed in black pods that were damaged by *E. ulicis* during the whole sampling period was 2.7 times higher at Stonehenge (45.5%) than at Lymington (16.7%) (Table 4.3). Similarly, on an annual basis there were also higher seed damage levels at Stonehenge than at Lymington. From August 2001 to July 2002, the percentage of all mature seed in black pods that were damaged by *E. ulicis* was almost twice as high at Stonehenge (34.4%) than at Lymington (18.1%). From April 2002 to March 2003, the percentage of all mature seed in black pods that were damaged by *E. ulicis* was more than four times as high at Stonehenge (55.5%) than at Lymington (12.4%) (Table 4.3).

**Table 4.2(a).** Number and percentage of seed damaged by *E. ulicis* in black pods on 30 gorse bushes at Stonehenge.

**Stonehenge**

	Date	No. bushes with black pods	No. black pods (no. infested)	No. damaged seed	No. undamaged seed	Percentage seed damage
W	15/8/01	0	0 (0)	0	0	
	29/8/01	0	0 (0)	0	0	
	12/9/01	0	0 (0)	0	0	
	19/9/01	0	0 (0)	0	0	
Spring	26/9/01	0	0 (0)	0	0	
	10/10/01	0	0 (0)	0	0	
	23/10/01	0	0 (0)	0	0	
	7/11/01	4	40 (9)	16	129	11
	21/11/01	8	65 (28)	67	152	30.6
	4/12/01	10	90 (46)	102	162	38.5
	18/12/01	8	70 (39)	93	91	50.4
	3/1/02	5	50 (18)	40	86	31.5
Summer	16/1/02	5	50 (18)	45	133	25.3
	30/1/02	2	20 (12)	36	44	44.7
	14/2/02	2	20 (9)	25	57	30.5
	28/2/02	1	10 (7)	18	11	61.4
	13/3/02	1	10 (8)	20	11	64.5
	27/3/02	0	0 (0)	0	0	
	10/4/02	0	0 (0)	0	0	
	24/4/02	0	0 (0)	0	0	
Autumn	7/5/02	0	0 (0)	0	0	
	22/5/02	0	0 (0)	0	0	
	5/6/02	0	0 (0)	0	0	
	19/6/02	0	0 (0)	0	0	
Winter	3/7/02	0	0 (0)	0	0	
	17/7/02	0	0 (0)	0	0	
	31/7/02	0	0 (0)	0	0	
	14/8/02	0	0 (0)	0	0	
	28/8/02	0	0 (0)	0	0	
	10/9/02	0	0 (0)	0	0	
	25/9/02	0	0 (0)	0	0	
	9/10/02	0	0 (0)	0	0	
Spring	23/10/02	0	0 (0)	0	0	
	6/10/02	0	0 (0)	0	0	
	20/11/02	0	0 (0)	0	0	
	3/12/02	2	20 (9)	28	33	45.5
	16/12/02	5	50 (38)	97	23	80.8
	3/1/03	17	170 (103)	276	237	53.8
	15/1/03	21	210 (113)	299	323	48
	29/1/03	6	60 (53)	132	28	82.5
Summer	12/2/03	1	10 (2)	5	28	15.2
	5/3/03	0	0	0	0	
	25/3/03	0	0	0	0	

**Table 4.2(b).** Number and percentage of seed damaged by *E. ulicis* in black pods on 30 gorse bushes at Lymington.

**Lymington**

	Date	No. bushes with black pods	No. black pods (n infested)	No. damaged seed	No. undamaged seed	Percentage seed damage
W	17/07/01	10	100 (0)	0	252	0
	31/07/01	10	100 (0)	0	264	0
Spring	14/08/01	10	100 (0)	0	235	0
	28/08/01	10	100 (0)	0	226	0
	12/09/01	11	110 (0)	0	242	0
	25/09/01	11	110 (0)	0	288	0
	09/10/01	8	80 (0)	0	216	0
Summer	22/10/01	7	70 (2)	8	203	3.8
	07/11/01	11	110 (26)	68	235	22.5
	20/11/01	10	100 (41)	82	206	28.6
	04/12/01	11	110 (49)	117	163	41.8
	19/12/01	9	90 (52)	133	109	55.0
Autumn	02/01/02	12	120 (62)	153	187	45.1
	15/01/02	16	160 (52)	104	331	23.9
	29/01/02	20	200 (88)	196	399	33.0
	12/02/02	16	160 (70)	178	296	37.5
	26/02/02	4	40 (17)	45	60	42.9
Winter	12/03/02	1	10 (3)	7	26	21.7
	26/03/02	0	0	0	0	
	09/04/02	1	10 (0)	0	36	0
	23/04/02	2	20 (0)	0	53	0
	07/05/02	6	60 (0)	0	145	0
Spring	21/05/02	8	80 (0)	0	199	0
	04/06/02	7	70 (0)	0	158	0
	18/06/02	10	100 (0)	0	266	0
	02/07/02	10	100 (0)	0	239	0
	16/07/02	13	130 (0)	0	335	0
Summer	30/07/02	13	130 (0)	0	295	0
	14/08/02	17	170 (0)	0	429	0
	27/08/02	17	170 (0)	0	436	0
	11/09/02	17	170 (0)	0	418	0
	24/09/02	17	170 (0)	0	417	0
Autumn	08/10/02	14	140 (0)	0	345	0
	22/10/02	12	120 (1)	2	300	0.7
	05/11/02	14	140 (15)	26	379	6.4
	19/11/02	7	70 (44)	92	105	46.8
	03/12/02	8	80 (53)	122	89	57.8
Winter	16/12/02	10	100 (62)	136	113	54.7
	02/01/03	4	40 (21)	55	48	53.4
	14/01/03	12	120 (29)	72	315	18.6
	28/01/03	5	50 (37)	89	45	66.4
	11/02/03	4	40 (27)	66	33	66.8
A	05/03/03	2	20 (16)	38	16	70.6
	25/03/03	0	0	0	0	

**Table 4.3.** Percentage of all green and black pods infested and percentage of all seed damaged by *E. ulicis* in two annual periods (August 2001 to July 2002 and April 2002 to March 2003) and over the entire 19 and 20 months of the study respectively at (a) Stonehenge and (b) Lymington.

**(a) Stonehenge**

<b>Period</b>	<b>Percent green pods infested (no. sampled)</b>	<b>Percent black pods infested (no. sampled)</b>	<b>Percent of all seed damaged in black pods</b>
August 2001 to July 2002	63.3 (1258)	45.5 (425)	34.4
April 2002 to March 2003	65.6 (1565)	61.2 (520)	55.4
August 2001 to March 2003	64.6 (2823)	54.2 (945)	45.5

**(b) Lymington**

<b>Period</b>	<b>Percent green pods infested (no. sampled)</b>	<b>Percent black pods infested (no. sampled)</b>	<b>Percent of all seed damaged in black pods</b>
August 2001 to July 2002	24.7 (3330)	19.6 (2370)	18.1
April 2002 to March 2003	26.8 (3407)	17.6 (2300)	12.4
July 2001 to March 2003	30.3 (5731)	18.4 (4160)	16.7



#### 4.4 Discussion

*E. ulicis* was responsible for a reduction in the amount of seed produced at both sites. However, reduced seed production does not necessarily translate into successful biological control (Crawley, 1989; Myers and Risley, 2000). It was estimated in a New Zealand population modelling study (Rees and Hill, 2001) that in order to cause a decline in gorse populations, it would be necessary to reduce the annual gorse seed crop by over 90% in conjunction with management practices that reduce seedling survival. Even though the levels of seed damage were higher at Stonehenge than at Lymington, the annual levels of seed damage obtained in this study (12.4-55.4%) were well below this level at both sites. Similarly, studies conducted in New Zealand (Hill *et al.*, 1991, Cowley, 1983) and Chile (Norambuena, 2000) have also found seed attack by *E. ulicis* to be well below the 90% level. Furthermore, *E. ulicis* by not attacking seeds in pods in a density dependent manner may compromise its effectiveness as a biocontrol agent as density-dependent foraging is considered a desirable biological attribute for classical biocontrol agents (van Driesche and Bellows, 1996). Therefore, increased levels of seed damage are required if biological control by seed feeders is to have an impact on gorse populations.

Another European seed feeder was released in New Zealand in 1992 and is now widely established. This species was originally identified as *Cydia succedana* Denis and Schifferrmüller (Lepidoptera: Tortricidae), but has since been identified as *C. ulicetana* Haworth (Q. Paynter, Landcare Research New Zealand, pers. comm., 2005). It has not been released in Australia (Ireson *et al.* 2004) as there is evidence that this species uses other legume species as alternative hosts to gorse (Fowler *et al.*, 2004). This species is bi-voltine and it was assumed by Hill and Gourlay (2002) that both

generations would attack gorse seed produced in spring and autumn in New Zealand. At one site in New Zealand, where seed was only produced in spring/summer similar to the Stonehenge site in this study, the combination of *E. ulicis* and *C. ulicetana* was reported to reduce seed production by 92% (T. R. Partridge, unpublished data cited by Hill and Gourlay, 2002). This was high enough to reduce the recruitment of gorse below replacement levels if management practises that reduce seedling survival were also used (Rees and Hill, 2001). However at a nearby site, where gorse plants only produced seed in autumn, seed production was only reduced by 8% (T. R. Partridge, unpublished data cited by Hill and Gourlay, 2002).

The underlying causes of the differences in the pod production patterns at the two Tasmanian sites were beyond the scope of this study. Biotic factors, such as genetic diversity between gorse populations, or abiotic factors, such as climatic differences between the sites, are possible explanations. Stonehenge is in a cooler inland region than Lymington, which is situated nearer the coast and experiences average annual temperatures almost 2°C warmer. Therefore, it is possible that the increased temperature at the Lymington site was at least partially responsible for the differences in the pod production patterns. However, further research would be required to test this hypothesis.

This study showed that poor synchronisation of *E. ulicis* larval activity with seed production of its host will be restricting the efficacy of *E. ulicis* at many Tasmanian sites. A higher proportion of seed escaped attack at the Lymington site where seed is produced outside the main period of larval activity. If temperature drive the patterns of gorse pod production, then this is probably a common occurrence at sites near the coast that experience warmer average temperatures. At the Stonehenge site, where *E.*

*ulicis* activity was closely synchronised with gorse pod production, a higher proportion of seed was attacked. This is probably a common occurrence at highland sites that experience cooler average temperatures. However, even at Stonehenge, the reduction in seed production due to *E. ulicis* alone was not enough to have an impact on gorse populations.

Clearly additional seed feeders would be required to further reduce seed production. However, a recent survey in the native range of gorse in Spain and Portugal (Sheppard, 2004), produced no evidence that there were any other autumn specific seed feeders active during this period. Even if species that don't attack a high proportion of the autumn seed crop exist, the contribution to seed damage in spring/summer could still be valuable at some sites. If the combination of the two seed feeding agents, *E. ulicis* and *C. ulicetana*, can reduce seed production by over 90% (as suggested by Hill and Gourlay, 2002) at sites that have similar pod production patterns to Stonehenge, then perhaps a reduction in populations of gorse will occur at such sites. At these sites, seed feeding biological control agents could therefore become an important component of a guild of agents that attack gorse at different stages of its lifecycle.

## **Chapter 5. The impact of gorse thrips, *Sericothrips staphylinus*, ryegrass competition, and simulated grazing on seedling performance of gorse, *Ulex europaeus*, in a controlled environment.**

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### **Abstract**

Biological control is often used in conjunction with other management practices to reduce the negative impacts of invasive weeds. When assessed in a factorial glasshouse experiment, a combination of gorse thrips, ryegrass competition, and simulated grazing (manual clipping) resulted in a gorse seedling mortality of 93% compared to no mortality in the untreated control. Mortality was 23% and 33% when ryegrass competition was combined with one additional factor of either thrips or grazing, respectively. Individual factors and the combination of gorse thrips and grazing did not cause any seedling mortality. The shoot dry weight of gorse seedlings was reduced by ryegrass competition (96%), simulated grazing (74%), and gorse thrips (57%). The root dry weight of gorse seedlings was also reduced by simulated grazing (70%) and gorse thrips (60%) but the effect of ryegrass competition was not measured. The interactions between treatments and the role of multiple control tactics within an integrated weed management program are discussed.

## 5.1 Introduction

A successful weed biological control agent should have a significant impact on the population dynamics of its host. As the time scale and resources may be limiting when attempting to quantify agent/weed dynamics, the success of biological control should only be claimed after it has been shown that the agent(s) have a measurable impact on aspects of the target weed's performance. Postrelease assessments should be conducted to quantify this impact and assess the competitive ability of the weed both with and without biological control agent(s). Postrelease impact studies are rarely conducted (Blossey and Skinner, 2000), hence such evaluation will also help improve the efficiency and effectiveness of subsequent weed biological control programs by identifying common factors affecting agent impact.

Integrated weed management is a multidisciplinary method using multiple, compatible control techniques to reduce weed populations below damaging levels. Biological control is often used in conjunction with other weed management practices as part of an integrated weed management program. Therefore, an understanding of the factors that contribute to the impact of biocontrol agents can greatly improve integrated weed management decisions by identifying options, such as chemical or cultural methods, available to land managers that enhance this impact (Farrell and Lonsdale, 1997). Interspecific competition from desirable plant species is recognized as an important factor contributing to the impact of biological control, especially in regard to invasive pasture weeds (Sheppard, 1996). Therefore a multidisciplinary approach is now being taken in weed management with recent studies targeting the interaction between a biological control agent and interspecific plant competition (for

examples see McEvoy *et al.*, 1993; Sheppard, 1996; Lonsdale and Farrell, 1998; Bacher and Schwab, 2000; Sheppard *et al.*, 2001).

The interaction between a biological control agent and interspecific plant competition falls into one of the following three categories outlined by Sheppard (1996): 1) *substitutive* interaction - the impact of one factor completely overwhelms the other; 2) *multiplicative* interaction - both factors have an impact but factors do not influence each other; and 3) *synergistic* interaction - impact of both factors working in conjunction is greater than the sum of each factor working independently.

Gorse, *Ulex europaeus* L. (Fabaceae), is native to Great Britain and central and western Europe, where it occurs in native heathland and neglected or disturbed farmland (Richardson and Hill, 1998). Gorse has been introduced to many countries worldwide and has become an important weed in Australia, New Zealand, Chile, Hawaii and in western regions of the USA. In Australia, gorse is a weed of national significance (Thorp, 1999), seriously affecting agricultural land and environmentally significant regions in South Eastern Australia.

Gorse is a spiny, leguminous, woody shrub which can live up to 30 years of age and grow to more than 3 m in height and diameter. Gorse forms dense, impenetrable monocultures that compete strongly with pasture (particularly in unimproved or semi-improved pastures) and other desirable plant species (Richardson and Hill, 1998). Flowering usually occurs in spring or early summer but the production of flowers outside this period, particularly in late autumn, is common. Seed is produced in small pods, often in large numbers, and can remain viable in soil in excess of 25 years (Moss, 1959). As gorse is such an abundant producer of long-lived seed, seedling

establishment and survival on disturbed ground is an important component of gorse population dynamics (Rees and Hill, 2001).

Pasture competition and grazing are recognized as important management practices commonly employed against gorse (Richardson and Hill, 1998). In typical pasture ecosystems, young gorse seedlings often compete with pasture species that are grazed by livestock. In glasshouse and field studies conducted in New Zealand, competition from pasture grasses reduced both the growth rate and survival of gorse seedlings, thus significantly suppressing their establishment (Ivens, 1979; Ivens and Mlowe, 1980). Furthermore, grazing by sheep in the field study and simulated grazing (by hand cutting) in the glasshouse study were found to further negatively affect the growth and survival of gorse seedlings with a negligible effect on the pasture species.

As part of an integrated management strategy, a suite of host-specific biological control agents are currently being investigated for gorse control in Australia (Ireson *et al.*, 1999). One of these, the gorse thrips, *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae), was introduced to Australia from New Zealand and released in January 2001. To date, this species has been released at 148 Tasmanian and 49 Victorian sites (Ireson and Kwong, unpublished data). Fowler and Griffin (1995) suggest that gorse thrips can reduce the growth of gorse seedlings and Hill *et al.* (2001) observed heavy damage to potted gorse plants under laboratory conditions with visible damage present at release sites in the field. This damage is caused by larval and adult gorse thrips feeding on mesophyll tissue. High numbers of gorse thrips produce distinct pale, stippled damage symptoms and abundant small frass droplets.

No detailed studies have been published on the phenology of gorse thrips under field conditions. However, Hill *et al.* (2001) studied their development under laboratory conditions at an average temperature of 19°C. At this temperature, female adults had an 8 to 12 day preoviposition period. White to pale yellow eggs were then laid in young plant tissue, these hatched after an average of 20 days. Gorse thrips then passes through two larval stages, a prepupal stage, and a pupal stage before adult emergence. The mean development time from egg to adult was 42 days and the life span of ovipositing adults was estimated to be 32.5 days with a lifetime fecundity averaging 76.2 eggs (Hill *et al.*, 2001).

It is likely that gorse thrips have the potential to reduce the growth and even survival of gorse seedlings when growing with a competitive pasture species. The aim of this study was to test the impact and define the interaction of gorse thrips, pasture competition, and simulated grazing on the growth and mortality of gorse seedlings in a glasshouse environment.



## 5.2 Materials and methods

### 5.2.1 Experimental design

‘Plots’ of six gorse seedlings were grown in each of 40 styrofoam boxes. These were set up as a factorial experiment in a randomised block design. Three factors, thrips (T), ryegrass competition (R), and simulated grazing (G), were used alone and in combination to give eight treatments replicated five times.

### 5.2.2 Trial establishment and maintenance

Gorse seeds were collected from a gorse infestation bordering the New Town laboratories, near Hobart, Tasmania. They were scarified by heating in an oven to 88°C for 30 minutes and then pregerminated by soaking in moist cotton wool for 4 days until the seed coat split. Gorse normally grows in a symbiotic relationship with *Rhizobium*, a root nodulating bacterium. An inoculating solution of this bacterium was prepared by grinding two grams of root nodules, collected from the roots of 3-year-old potted gorse plants, with a mortar and pestle and mixing with 200 ml of distilled water. This solution was then immediately misted onto the germinating seed. Finally, the pregerminated and inoculated seeds were sown into seedling trays and maintained at approximately 20°C in a temperature-controlled glasshouse. The trays were hand-watered as required.

Once seedlings reached an average height of  $1.9 \pm 0.1$  cm (day 0) they were randomly allocated into treatments, and transplanted into 45 × 29 × 18 cm styrofoam boxes. Six seedlings were planted per box in two rows of three with a minimum spacing of 10 cm between each seedling. Each box contained a fertilizer-amended potting mix of coarse sand, composted pine bark, and coco-peat in a ratio of 5:4:1. The trial was

conducted in a temperature-controlled glasshouse maintained at approximately 20°C with a 16-h photoperiod.

The following day, seeds of a competitive pasture species, (*Lolium perenne* L. cv. Vic), were sown around the gorse seedlings at a rate of 1.5 g per m<sup>2</sup> into the seedling boxes representing ‘ryegrass competition’ treatment. This rate is equivalent to a commonly used commercial field rate of 15 kg per ha. Once the gorse seedlings and pasture were well-established (29 days after transplanting), gorse thrips from a glasshouse culture were added to the ‘thrips’ treatments at a rate of 10 adult thrips per gorse plant. Gorse seedlings had a mean height ( $\pm$  SE) of  $5.5 \pm 1.7$  cm at the time of thrips introduction. Simulated grazing was conducted 47 days after gorse was transplanted in the seedling boxes of the ‘grazing’ treatment by trimming the gorse or gorse and pasture mix with scissors to 3 cm in height and removing clippings.

The thrips-free treatments were inspected every 3-4 days to assess the plants for the presence of thrips. To ensure that the number of thrips moving onto the thrips-free plants was kept to a minimum, the systemic insecticide omethoate (a.i. 2 g/kg 0,0-dimethyl S-methylcarbamoylmethyl phosphorothioate) was applied in an aerosol form (Folimat®) on two occasions (37 and 94 days following transplanting). Two applications of omethoate did not significantly alter the final plant dry weight of potted gorse seedlings in a pot trial conducted concurrently (Davies, unpublished data).

### 5.2.3 Harvest and assessment

Harvest commenced 123 days after transplanting gorse seedlings. All gorse plants were sequentially harvested over a period of 12 days and thrips were collected by

extraction from one replicate at a time. Three plants from the total of six within each box were randomly selected for thrips extraction and counts. To extract thrips, plants were placed in 12.5-cm-diameter Tullgren funnels for 3 days using 25 watt incandescent lights 25 cm from the grids. Adult and juvenile thrips were collected into 120 ml plastic tubes containing 30 ml of 70% alcohol and a drop of glycerol. Collected thrips were counted in a petri plate at 15× magnification under a dissecting microscope. Shoot material was harvested at the cotyledon level before assessment. Finally, all plants were oven-dried for 3 days at 70°C before weighing.

The thick mass of ryegrass roots made the removal of gorse roots impractical. Therefore, roots were only harvested and washed in treatments without ryegrass competition. This reduced the number of treatments for this assessment from eight to four. All harvested root material was oven-dried for 3 days at 70°C before weighing.

#### *5.2.4 Data analysis*

All statistical tests were performed using GENSTAT 6<sup>th</sup> edition (VSN Int., Ltd., 2002). The proportion mortality was calculated for gorse seedlings within plots, this data was arcsine square-root transformed and subjected to ANOVA. Dry shoot and root weights per individual plant were square-root transformed and subjected to ANOVA. Thrips numbers per gram of shoot dry weight (sdw) were calculated for each plant, natural log (+0.005) transformed, then subjected to ANOVA. For this analysis, zero values for seedlings that did not survive and low sdw values for plants that were considered too unhealthy to support gorse thrips were excluded. To separate means in figures 1-4, least significant differences (LSD) were calculated from the appropriately transformed data. All data presented in figures 1-4 were plotted using untransformed means.

Changes in the mean percentage dry weights of shoots and roots (except ryegrass competition) and mean percentage change in thrips numbers per gram of dry shoots were calculated for each factor of thrips, grazing, and ryegrass competition across all eight treatments. Treatment means from each replicate were initially calculated. The mean percentage change due to each factor was then calculated across each replicate using each treatment (Tr) with a corresponding untreated counterpart (UTr):

$$\% \text{ change} = (\text{mean UTr} - \text{mean Tr} / \text{mean UTr}) \times 100$$

For example, if calculating the percentage change due to thrips in each of the following treatments, T, TG, TR, and TGR, the untreated counterparts were control, G, R, and GR, respectively. Finally, a total mean percentage change for each factor was calculated by averaging each of the individual percentage changes across all treatments.

Many gorse seedlings in the TGR treatment either did not survive or became severely stressed. These seedlings had very low thrips numbers at the time of sampling, probably due to a reduction in plant quality. The TGR treatment and its untreated counterpart were therefore excluded from the calculation for the change in thrips numbers per gram of dry shoots.

## Results

### *5.3.1 Mortality of gorse seedlings*

Thrips, grazing, and ryegrass competition all had a significant effect on gorse seedling mortality (Table 5.1). However, as it was a factorial experiment, this effect was due to factor combinations (TR, GR and TGR) rather than the individual factors themselves. There was no mortality in the untreated control, any of the single factor treatments, or the combination of thrips and grazing. Only those treatments that combined ryegrass competition with either thrips or grazing increased the percentage mortality of gorse seedlings compared to the untreated control (Fig. 5.1). When the ryegrass competition was combined with either thrips or grazing, mortality was  $23 \pm 6\%$  and  $33 \pm 9\%$  respectively. Furthermore, when all three factors (TGR) were combined, mortality increased to  $93 \pm 7\%$  (Fig. 5.1). The interaction between thrips and ryegrass competition and grazing and ryegrass competition were significant at the 0.001 level (Table 5.1). The interactions between thrips and grazing and thrips, grazing and ryegrass competition were not significant.

### *5.3.2 Gorse biomass production*

Individually, the thrips, grazing and ryegrass competition all significantly reduced the shoot dry weight of gorse seedlings when compared with the untreated control (Fig. 5.2, Table 5.1). Across all treatments the thrips reduced shoot dry weight by an average of 57%, grazing by an average of 74%, and ryegrass competition by an average of 96%. There were significant interactions between treatments when ryegrass competition was combined with either thrips or grazing, however, no significant interaction was found between grazing and thrips or the combination of all

three factors (Table 5.1). When compared to the untreated control, a combination of grazing and thrips reduced shoot dry weight by 87%, thrips and ryegrass competition by 98%, and grazing and ryegrass competition by 99%. All three factors combined reduced the shoot dry weight by close to 100% (Fig. 5.2).

Although gorse roots from the ryegrass competition treatments could not be weighed, thrips and grazing both significantly reduced root dry weights (Fig. 5.3, Table 5.1). On average the thrips treatment reduced dry root weight by 60% and the grazing treatment by 70%. Although the combination of thrips and grazing reduced root dry weight by 89% compared to the untreated control, the interaction between thrips and grazing was not significant (Table 5.1, Fig. 5.3).

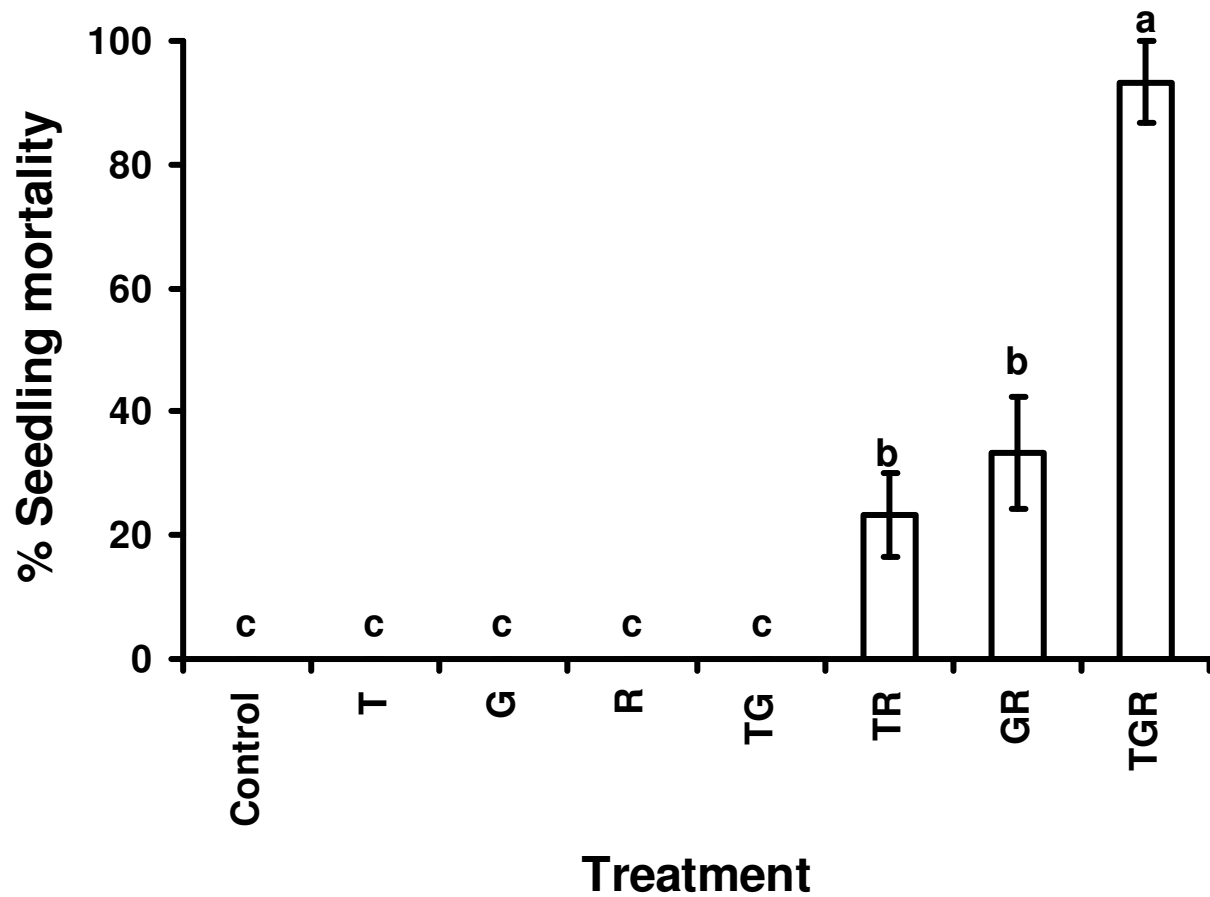
### *5.3.3 Effects of treatments on gorse thrips numbers*

Across all treatments there were significantly more gorse thrips extracted from thrips-treated plants (30 thrips per gram of shoot dry weight) than their untreated counterparts (3 thrips per gram of shoot dry weight) (Fig. 5.4, Table 5.1). There were also significantly more thrips extracted per gram of dry shoot weight from both the grazing and ryegrass treatments (Table 5.1). Across all treatments, grazing increased the number of thrips per gram of shoot dry weight by a factor of 2.3 and ryegrass competition increased the number of thrips by a factor of 2.7 compared to their respective untreated counterparts. There were no significant interactions between any combination of two factors (TG, TR, GR) or the combination of three factors (TGR) (Table 5.1).

**Table 5.1.** ANOVA *F* statistics and *P*-values for effects of thrips (T), grazing (G), and ryegrass competition (R) on gorse seedling mortality, shoot dry weight (shoot DW), root dry weight (root DW) (excluding rye treatment combinations), and thrips numbers per gram of shoot dry weight.

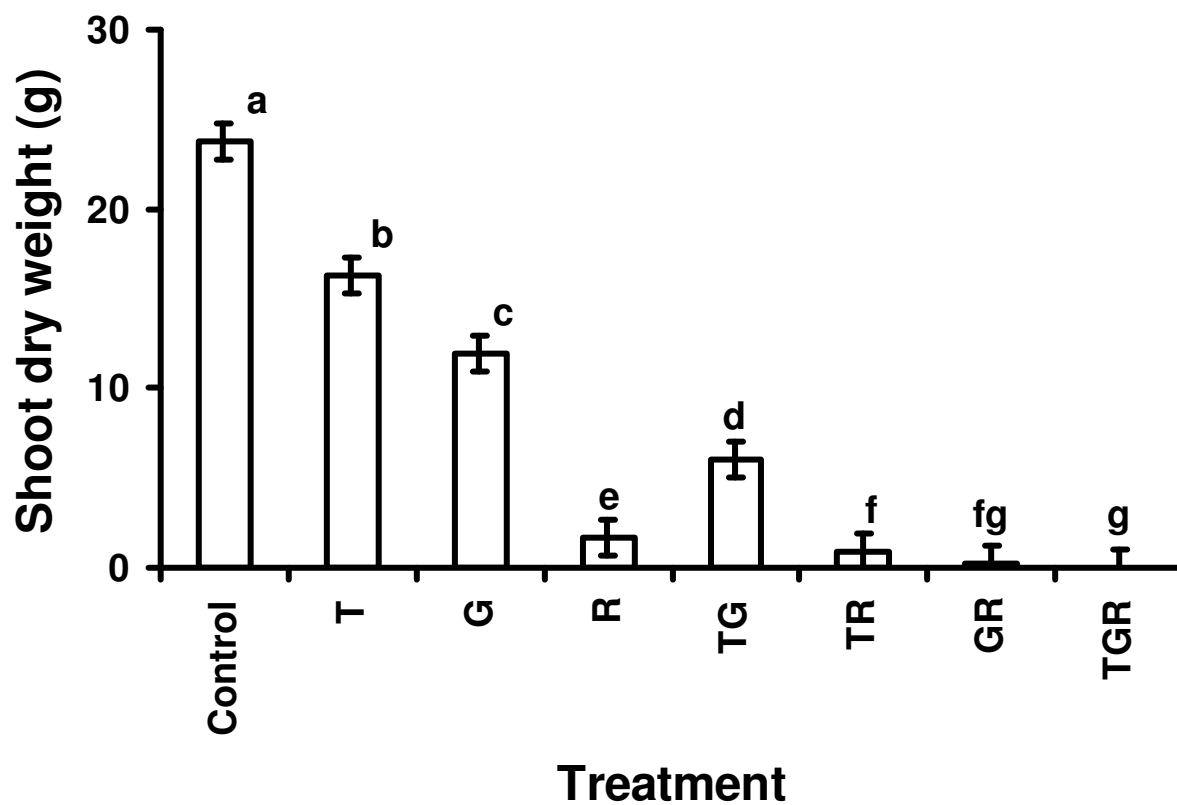
Source of variation:	Mortality (1) <sup>a</sup>		Shoot DW (2)		Root DW (2)		Thrips no.s (3)	
	<i>F</i> <sub>1,28</sub>	<i>P</i>	<i>F</i> <sub>1,28</sub>	<i>P</i>	<i>F</i> <sub>1,12</sub>	<i>P</i>	<i>F</i> <sub>1,22</sub>	<i>P</i>
Thrips (T)	42	<0.001	34	<0.001	33	<0.001	131	<0.001
Grazing (G)	65	<0.001	98	<0.001	56	<0.001	23	<0.001
Rye (R)	136	<0.001	671	<0.001	N/A	N/A	18	<0.001
T × G	8	0.057	0	0.96	0.7	0.43	0.6	0.45
T × R	42	<0.001	4.6	0.041	N/A	N/A	1.2	0.28
G × R	64.7	<0.001	8.4	0.007	N/A	N/A	1.9	0.18
T × G × R	8.1	0.057	0.3	0.58	N/A	N/A	3.4	0.08

<sup>a</sup> Data were transformed as follows: (1) = arcsine square root transformation; (2) = square-root transformation; (3) = log<sub>e</sub>(thrips/grams shoot dry weight + 0.005).

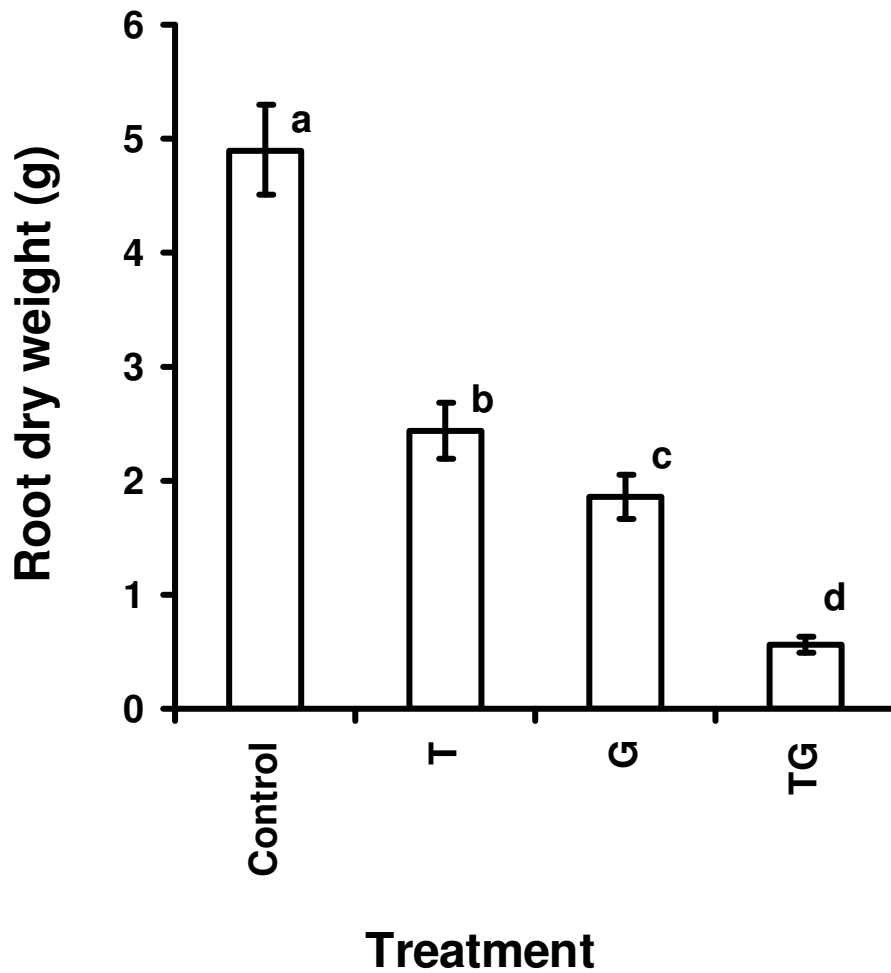


**Figure 5.1.** Mean ( $\pm$  SE) percentage mortality of gorse seedlings exposed to combinations of a thrips treatment (T), a grazing treatment (G), and a ryegrass competition treatment (R). Each treatment consisted of 30 gorse seedlings: 6 gorse seedlings per box and 5 replicate boxes. Means with the same letter are not significantly different at 0.05 level (LSD calculated on arcsine square root transformed individual plant data).

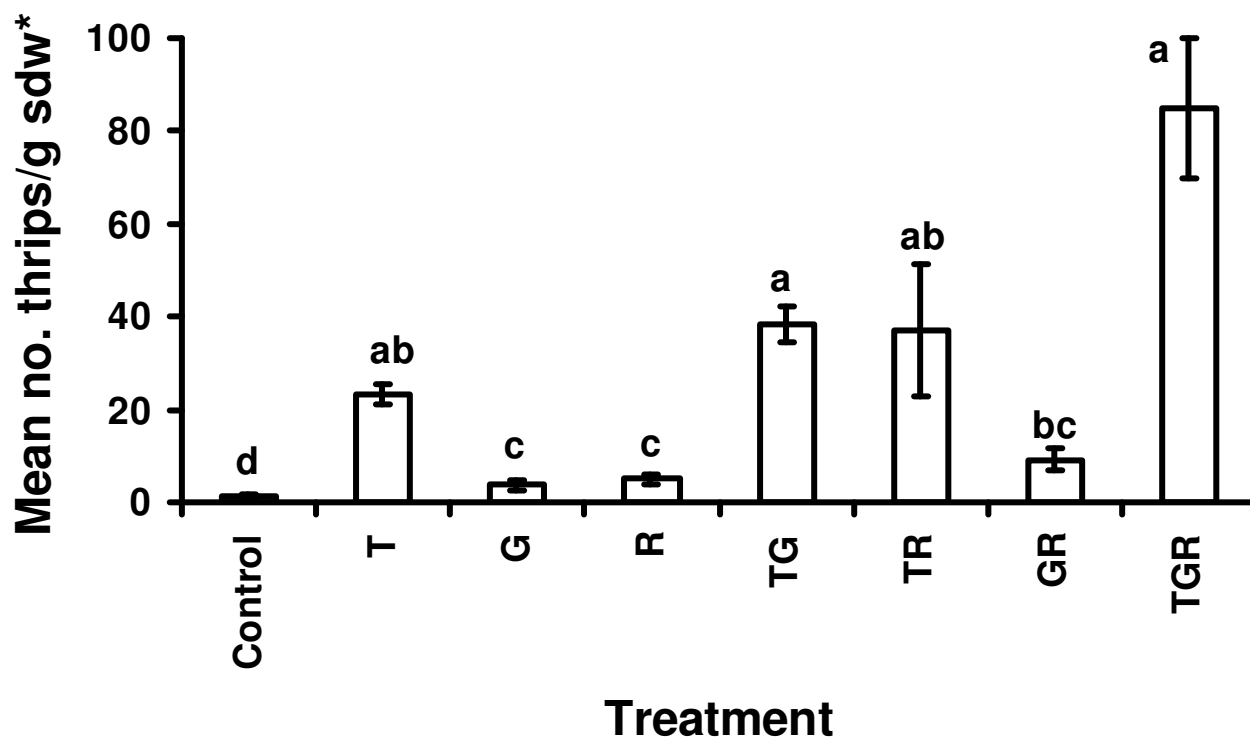




**Figure 5.2.** Mean ( $\pm$  SE) shoot dry weight of gorse seedlings exposed to combinations of a thrips treatment (T), a grazing treatment (G), and a ryegrass competition treatment (R). Each treatment consisted of 30 gorse seedlings: 6 gorse seedlings per box and 5 replicate boxes. Means with the same letter are not significantly different at 0.05 level (LSD calculated on square root transformed individual plant data).



**Figure 5.3.** Mean ( $\pm$  SE) root dry weight of gorse seedling roots exposed to combinations of a thrips treatment (T), a grazing treatment (G), and a ryegrass competition treatment (R). Each treatment consisted of 30 gorse seedlings: 6 gorse seedlings per box and 5 replicate boxes. Means with the same letter are not significantly different at 0.05 level (LSD calculated on square root transformed individual plant data).



**Figure 5.4.** Mean ( $\pm$  SE) number of gorse thrips (*Sericothrips staphylinus* – immatures and adults) per gram of shoot dry weight (sdw\*) extracted by tullgren funnel from 3 of 6 randomly selected gorse seedlings per box. Plants that did not survive or those considered too unhealthy to support thrips populations were excluded. Means with the same letter are not significantly different at 0.05 level. LSD's were calculated on natural log (thrips per gram sdw + 0.005) transformed individual plant data.

## 5.4 Discussion

The importance of multiple control tactics within an integrated weed management program was highlighted in this study. Although single factors did not cause any gorse seedling mortality, combinations of two and three factors did. As seedling survival is an important component of gorse population dynamics (Rees and Hill, 2001), any management strategy or combination of strategies that reduces seedling survival will be important. If similar combinations of gorse thrips, competition from pasture species, and grazing management also affect gorse seedling survival under field conditions, then the gorse thrips could become an important component of an integrated management strategy, especially in the earlier stages of gorse establishment.

This study has also demonstrated that thrips, grazing, and ryegrass competition can independently reduce the biomass of gorse seedlings in a glasshouse environment. Although the impact of the gorse thrips was less than the other treatments, it is widely accepted that even small reductions in growth of one plant species can result in major shifts within the competitive balance of plant communities (Crawley, 1989).

The interaction between biological control and interspecific plant competition is most often multiplicative and infrequently synergistic or substitutive (Sheppard, 1996). As there was a significant interaction between the thrips and ryegrass competition in this study, a synergistic interaction, where the impact of one factor has increased the impact of the other, was found to occur through an increase in seedling mortality and a reduction in shoot dry weight.

Individually, interspecific plant competition affects only the plant growth rate and a phytophagous biological control agent affects the plant loss rate (Crawley, 1989). Therefore, in a synergistic interaction where the combined impact is greater than the additive impact of

each factor working independently, the biological control agent will also affect the plant growth rate due to the increased impact of competition.

As this study was conducted in a controlled environment, further research is required to determine if this type of interaction would occur in a field situation where other factors may influence thrips and gorse population dynamics. These other factors may include the influence of natural enemies on thrips populations, thrips dispersal behaviour, and plant quality. Local climatic conditions may also have major influences on thrips population dynamics and on gorse and pasture growth. As all three may develop at different rates under field conditions, the potential impact of both the thrips and pasture competition may vary. Even if the synergistic interaction is not found in a field situation and the more common multiplicative interaction is found where neither factor influences the other, such an effect should still be a positive outcome for the management of gorse. It is also possible that the gorse thrips may affect the seed production capability and/or longevity of mature plants. Future studies could also investigate the impact of gorse thrips on the reproductive capability and longevity of mature gorse.

In addition to pasture and grazing management, a range of other management practices are employed against gorse including application of herbicides, fire, mulching and mechanical clearing (Richardson and Hill, 1998). Other biological control agents are also under investigation in Australia (Ireson *et al.*, 2004). As part of an overall integrated management strategy for gorse, future field studies on the management of this weed will need to consider the impact and compatibility of the additional management practices available including the use of other biological control agents.

## Chapter 6. Potential natural enemies of *Sericothrips staphylinus* within the arthropod fauna inhabiting gorse, *Ulex europaeus*.

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### Abstract

Natural enemies can regulate populations of phytophagous arthropods. In weed biological control programs, natural enemies may reduce the impact of biological control agents on the target weed. A field study was conducted over 21 months in southern Tasmania, Australia to determine if the composition of the arthropod fauna on gorse contained potential natural enemies of *Sericothrips staphylinus*. A range of potential arthropod natural enemies were identified during this study. These consisted mainly of generalist predators, which could possibly reduce the impact of *S. staphylinus* and provide an explanation for its slow dispersal rate. High levels of another herbivorous thrips species *Odontothripsella* sp., may act as a significant prey item for the generalist predators, and were significantly correlated with the stages associated with gorse flowering. The Phlaeothripid *Haplothrips victoriensis* and mites in the family Phytoseiidae were present on gorse throughout the study and are reported to be natural enemies of other members of the family Thripidae. *H. victoriensis* were most significantly correlated with the abundance of green pods on bushes whereas phytoseid mites were strongly correlated with the number of adult *H. victoriensis*. Further studies are required to test the predatory efficacy of these arthropods on *S. staphylinus*.

## 6.1 Introduction

Host specific phytophagous arthropods are often used as biological control agents for invasive weeds. Natural enemies often play a key role in regulating populations of phytophagous arthropods and are extensively utilised for the biological control of agricultural and horticultural pests. However, in weed biological control, natural enemies may reduce the population size and therefore the effectiveness of weed biocontrol agents (Goeden and Louda, 1976; McFayden and Spafford-Jacob, 2004).

Gorse, *Ulex europaeus* L. (Fabaceae), is a leguminous European woody shrub that has become invasive in many temperate regions of the world. In Australia, gorse is a weed of national significance (Thorp, 1999) seriously affecting agricultural and environmentally significant regions in South Eastern Australia. As part of an integrated management strategy, a guild of host-specific biological control agents are currently being introduced to gorse in Australia (Ireson *et al.*, 2004).

The gorse thrips, *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae), was introduced to Australia from New Zealand and released in 2001 (Ireson *et al.* 2004). In a glasshouse environment this species reduced the growth of gorse seedlings and also reduced seedling survival when combined with other management practices (see Chapter 5). Although populations of *S. staphylinus* can increase to high densities in the protected environment of a glasshouse, only low densities have been recorded under field conditions and dispersal has been recorded at no more than three metres from the parent bush four years after release (Ireson *et al.*, 2006). Natural enemy attack could be one of the factors responsible for maintaining populations at low densities.

The aim of this study was to determine if potential natural enemies of *S. staphylinus* were present within the arthropod fauna inhabiting gorse in southern Tasmania.



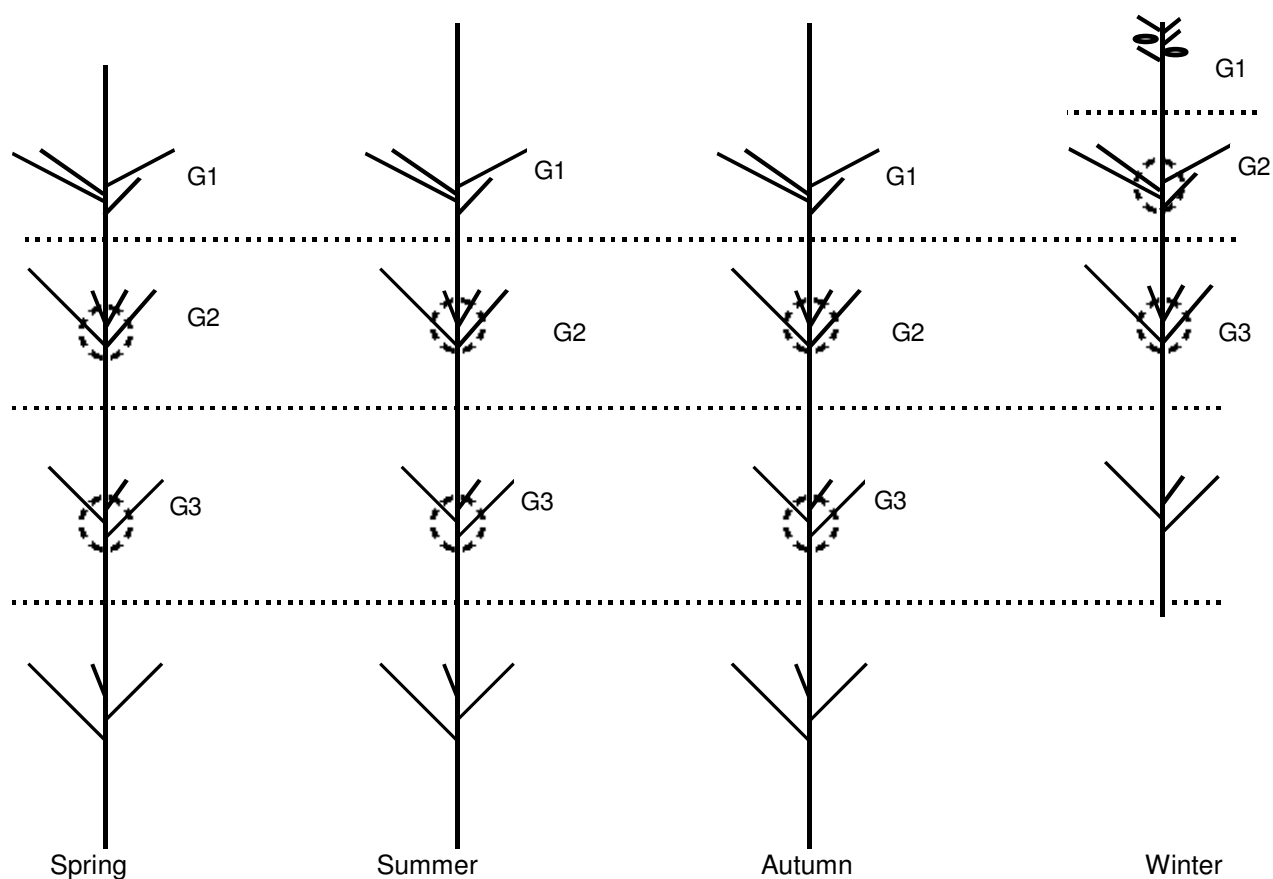
## 6.2 Materials and Methods

### 6.2.1 The study site

The study was conducted within a gorse infestation on a hillside with a north-easterly aspect located on a hobby farm near Lymington, Tasmania (43° 11' S, 147° 01' E). The gorse was growing amongst introduced pasture species and adjacent to native tree species (eg. *Acacia dealbata* and *Eucalyptus* spp.). Gorse bushes at this site were approximately seven years of age and between one and three metres in height and diameter. *S. staphylinus* was released at two time periods, 21 and 18 months prior to the commencement of this study respectively, in summer and late autumn 2001 (7/2/2001 and 25/5/2001 respectively) (J. E. Ireson, unpublished data). The summer release was of 300 adult *S. staphylinus* and the late autumn release was an open release using potted culture plants. Collectively these plants held well over 2000 adult and juvenile *S. staphylinus*.

### 6.2.2 Gorse sampling procedure

Six areas of easily accessible gorse were randomly chosen and tagged, to mark four by four metre sampling regions. As dispersal and spread is so limited, all sampling regions were as close as possible, but no more than 15 metres from the original *S. staphylinus* release. A single gorse plant was haphazardly selected from within each sampling region every two to four weeks for 21 months between November 2002 and July 2004 for sampling on each date. To ensure that a range of microhabitats were sampled (including flowers/fruit, new foliage and older growth), plant material was collected from upper, mid and lower parts of the plants, which were designated as G1, G2 and G3 respectively (Fig. 6.1).



**Figure 6.1.** Diagrammatic representation of the seasonal sampling regions of gorse over time. The G1 region was the top 15 cm of the previous seasons growth plus all new growth (including flowers and pods) arising from this. The G2 and G3 regions were the branch junctions from the main stem from one and two previous seasons respectively. To collect these, all branches were pruned off the branch junction in a seven centimetre radius from the central branch junction.

As the gorse plants underwent significant seasonal changes in their morphology over the sampling period, the size of the G1 samples also varied. When the study was initiated in spring 2002, the G1 region was the top 15 cm of the previous seasons growth plus all new growth (including flowers and pods) that arose from this (Fig. 6.1). As the season progressed, the same regions of the plant were collected but plants grew so the size of individual G1 samples became larger. When growth had ceased in winter 2003, the G1 regions again became the top 15 cm of the previous seasons

growth. In spring 2003 the G1 region was again the top 15 cm of the previous seasons growth plus all new growth (including flowers and pods) that arose from this.

Similarly, in summer and autumn the G1 region was again the top 15 cm of the previous seasons growth plus all new growth.

The G2 and G3 regions were the branch junctions from the main stem from one and two previous seasons respectively (Fig. 6.1). Unlike the G1 sampling regions, these were of a similar size irrespective of season. To collect these samples, all branches were pruned off the branch junction in a seven centimetre radius from the central branch junction. This resulted in a sample 14 cm in diameter consisting of branch stubs, some green tissue on the lower end of branches (in G2 samples only) and trapped leaf litter.

To document changes in the plants over the sampling period, the growth and reproductive status of each G1 sampling region were assessed. To estimate the amount of vegetative growth, the average shoot length was first determined by measuring four random shoots on the sample. This was then multiplied by the number of shoots then added to the original 15 cm of the previous seasons growth. To assess the reproductive status of each G1 sample, the number of each of the following flower/pod stages were counted:

- ◆ Flower buds – greater than 2 mm in diameter and petals not visible through sepals.
- ◆ Flowers – petals visible through sepals but no pod visible.
- ◆ Green pods – tip of pod above sepals, more than 50% of the pod green in colour.
- ◆ Black pods – greater than 50% of pod black in colour.

### 6.2.3 Arthropod sampling procedure

All plant material collected per sampling region was then roughly cut into pieces approximately 2-4 cm in length and placed into 12.5-cm-diameter Tullgren funnels for 3 days using 25 watt incandescent lights 25 cm from the grids. Cutting of each plant sample was conducted over a large, clean white plastic tray so that any arthropods that were displaced during this process would not be lost. Arthropods were collected into 120 ml plastic tubes containing 30 ml of 70% alcohol and a drop of glycerol.

Samples of mites (Acari) and thrips (Thysanoptera) were cleared in Konos solution (Jeppson, *et al.*, 1975) and slide mounted in Berlese medium (Swan, 1936). All other arthropods were identified from alcohol preserved specimens. All arthropods, except for microhymenoptera (several families in the hymenopteran suborder Apocrita), were identified to family. Insects were identified using the relevant keys in Naumann (1991) and mites (Acari) using keys in Krantz (1978).

Species of Thysanoptera in the families Thripidae and Phlaeothripidae were identified to genus using keys in Mound and Gillespie (1998) and Palmer, *et al.*, (1989). For further identification of *Odontothripiella* and *Haplothrips*, voucher specimens were forwarded to L.A. Mound, CSIRO Entomology, ACT, Australia.

Mites (Acari) in the family Phytoseiidae were identified to genus and/or species using keys in Chant and McMurtry (1994) and Beard (2001). Mites (Acari) in the families Ascidae, Bdellidae and Cunaxidae were identified to genus using keys in Halliday, *et al.*, (1998), Wallace and Mahon (1976) and Smiley (1992) respectively.

Those families (and microhymenoptera) that included other arthropods in their diet (Naumann, 1991; Krantz, 1978) were classified according to their feeding habits: generalist predators - feed across a range of arthropod genera; specialist predators - feed on arthropods within a genus; parasitoids - feed and develop within an arthropod host resulting in its death; omnivores - feed on both arthropods and plant material. These arthropods and *S. staphylinus* were counted in a petri plate at 15× magnification under a dissecting microscope. Numbers presented in Table 6.2 are the total numbers collected from all G1, G2 and G3 regions from all six sampling regions.

#### 6.2.4 Data analysis

Correlation analyses were conducted to determine the relationship between plant factors (vegetative shoot growth, buds, flowers, green pods and black pods) and herbivorous or omnivorous arthropods (*S. staphylinus* adults, *S. staphylinus* juveniles, *Odontothripiella* adults, *Odontothripiella* juveniles, *H. victoriensis* adults, *H. victoriensis* juveniles). A further correlation analyses was conducted to determine if there was any relationship between prey (*S. staphylinus* adults, *S. staphylinus* juveniles, *Odontothripiella* adults and *Odontothripiella* juveniles, *H. victoriensis* adults, *H. victoriensis* juveniles) and potential natural enemies (*H. victoriensis* adults, *H. victoriensis* juveniles, Phytoseiids, Anystids, Erythraeids, Cheyletids, and microhymenoptera). Bonferroni corrected probabilities were used for all correlations thereby accounting for multiple comparisons.

### 6.3 Results

Only those arthropods that were deemed to be potential natural enemies of *S. staphylinus* are presented in this study. A number of potential natural enemies were collected from gorse during the course of this study (Table 6.1). Of these, members of the families Phlaeothripidae, Thripidae, Phytoseiidae, Cheyletidae, Anystidae and Erythraeidae are reported as predators of members of the family Thripidae (Sabelis and Van Rijn, 1997), of which the biocontrol agent *S. staphylinus* is a member. In addition, hymenopteran egg and larval parasitoids of the superfamily Chalcidoidea also attack members of the family Thripidae, (Loomans, *et al.*, 1997). Many species of Chalcidoidea are microhymenopterans. Mites in the family Ascidae, Bdellidae and Cunaxidae were also found and are known as predators of arthropods (Krantz, 1978). There are no reports of any member of these families preying on members of the family Thripidae.

**Table 6.1.** Potential arthropod natural enemies collected from gorse at Lymington between spring 2002 and winter 2004.

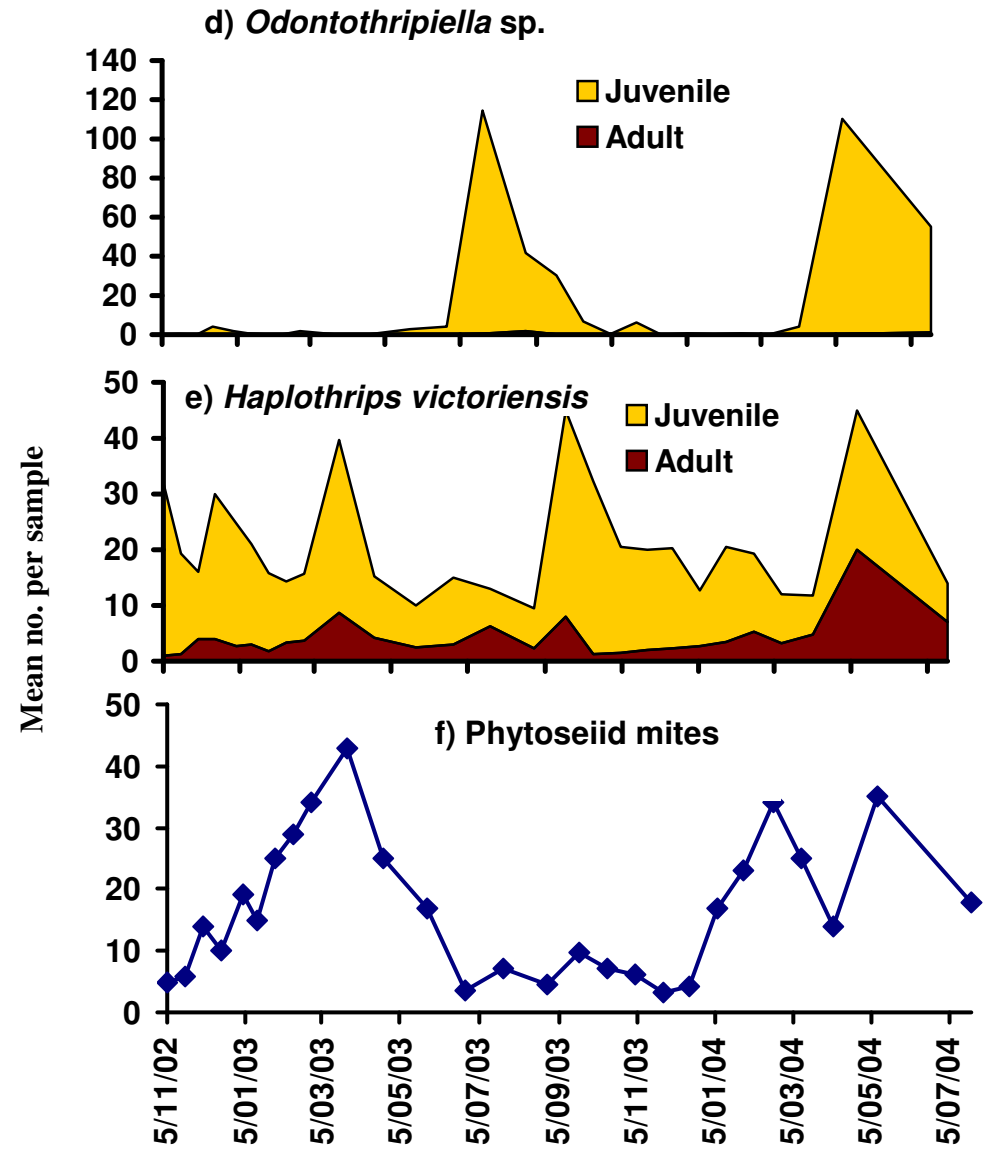
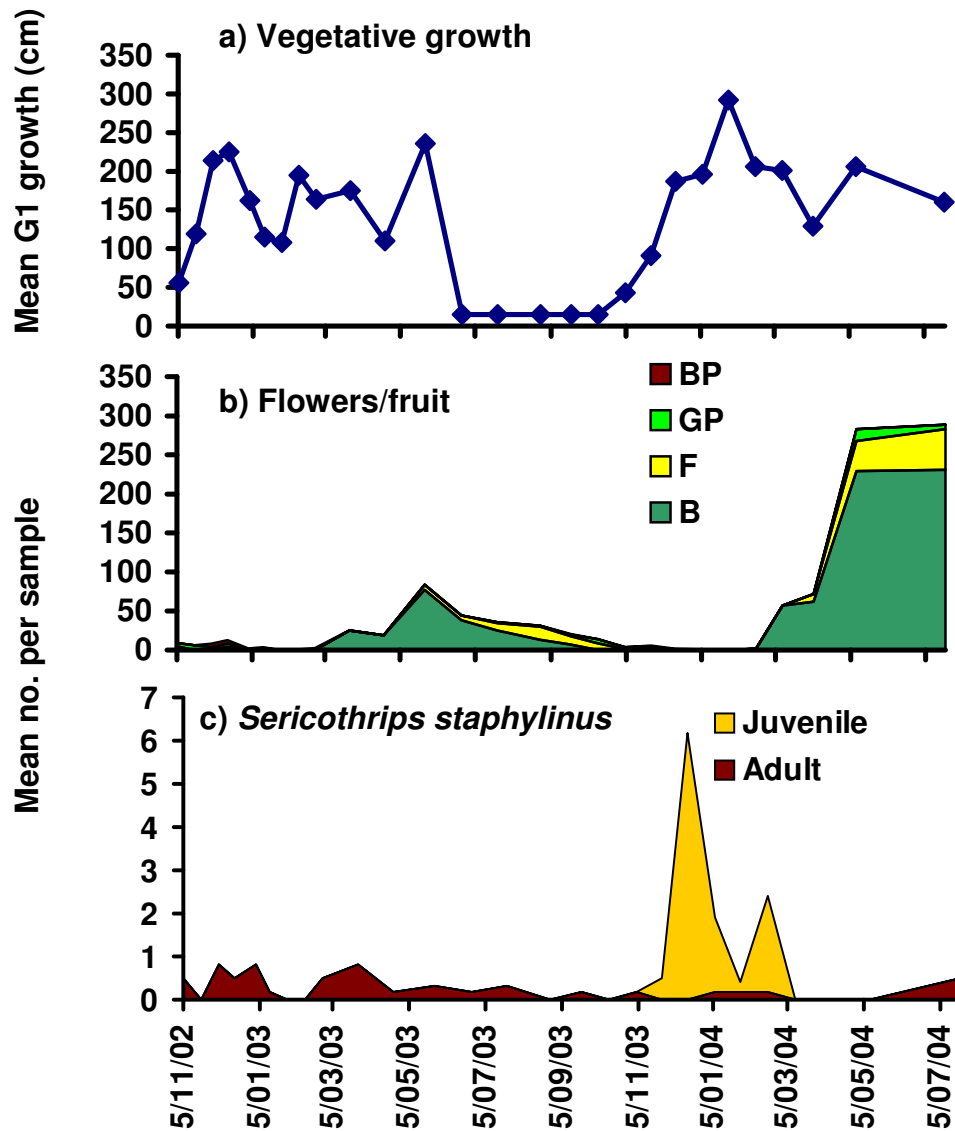
Arthropods			Natural enemy classification <sup>1</sup>			
			GP	SP	Om	Par
Insects:	Phlaeothripidae <sup>2</sup>	<i>Haplothrips victoriensis</i> (Bagnall)	✓		✓	
	Thripidae <sup>2</sup>	<i>Odontothripella</i> sp.			✓	
	Microhymenoptera <sup>2</sup>					✓
Mites:	Tydeidae		✓		✓	
	Phytoseiidae <sup>2</sup>	<i>Typhlodromus helenae</i> (Schicha and Dosse) <i>Proprioseiopsis</i> sp.	✓	✓		
	Cheyletidae <sup>2</sup>		✓			
	Anystidae <sup>2</sup>		✓			
	Erythraeidae <sup>2</sup>		✓			
	Ascidae	<i>Asca</i> sp.	✓		✓	
	Bdellidae	<i>Bdella</i> sp., <i>Bdellodes</i> sp.	✓			
	Cunaxidae	<i>Cunaxa</i> sp.	✓			

<sup>1</sup>GP = generalist predator; SP = specialist predator, Om = Omnivore, Par = parasitoid.

<sup>2</sup>Members of these families (and microhymenoptera) are known to be natural enemies of members of the family Thripidae.

Vegetative growth of gorse occurred over all months but for the winter whereas flowering of gorse occurred mainly in late autumn, winter and spring (Table 6.2, Fig. 6.2a, 6.2b). Shortly after flowering, green and then black pods were produced. The numbers of *S. staphylinus* and those arthropods deemed to be potential natural enemies of *S. staphylinus* are also shown in Table 6.2 and Fig. 6.2c-f.

*S. staphylinus* adults were present almost all year but the juveniles were only present in spring and summer with their numbers very low and not significantly correlated to any measured plant factor (Fig. 6.2c). However, average densities of both adults and juveniles were only recorded at low levels (< 2.2 per plant sample with the exception of one sample date) throughout the study (Table 6.2).



**Figure 6.2.** Gorse sample details: **a)** Vegetative growth (mean length of G1 growth sampled) and **b)** Mean number of flowers and fruit (B = buds, F = flowers, GP = green pods and BP = black pods) and mean number of arthropods per sample: **c)** *Sericothrips staphylinus*, **d)** *Odontothripiella* sp., **e)** *Haplothrips victoriensis* and **f)** Phytoseiid mites.



**Table 6.2.** Gorse sample details and numbers of *S. staphylinus* and potential natural enemies of *S. staphylinus* extracted from gorse at Lymington using Tullgren funnels over 21 months of sampling between spring (Sp) 2002 and winter (W) 2004.

	Date	Gorse sample details (mean $\pm$ SE) <sup>1</sup>							Mean ( $\pm$ SE) number of Arthropods <sup>2</sup>								
		VG	B	F	GP	BP	Ss A	Ss J	Osp A	Osp J	Hv A	Hv J	Phyto	Anys	Ery	Chey	Michy
Sp	5/11/02	56 $\pm$ 14	0	5 $\pm$ 5	3.8 $\pm$ 3.8	0	0.5 $\pm$ 0.5	0	0	0	1 $\pm$ 0.4	31 $\pm$ 23	5 $\pm$ 3.3	0	0	0	0
	19/11/02	119 $\pm$ 65	0	0	6 $\pm$ 4.8	0	0	0	0	0.3 $\pm$ 0.3	1.3 $\pm$ 0.6	18 $\pm$ 6.5	6 $\pm$ 2.4	0	0	0	0
	3/12/02	214 $\pm$ 38	0	1.5 $\pm$ 0.9	2.3 $\pm$ 1.4	4.3 $\pm$ 2.3	0.8 $\pm$ 0.3	0	0	0	4 $\pm$ 2.1	12 $\pm$ 2.4	14 $\pm$ 3.4	0	0.3 $\pm$ 0.3	0	0
	16/12/02	225 $\pm$ 61	0	4 $\pm$ 2.7	4 $\pm$ 2.6	4.7 $\pm$ 4.1	0.5 $\pm$ 0.2	0	0	4 $\pm$ 2.6	4 $\pm$ 1.4	26 $\pm$ 6.2	10 $\pm$ 2.1	0.2 $\pm$ 0.2	0	0	0
Sum	2/1/03	162 $\pm$ 12	0	0	0.7 $\pm$ 0.5	1.2 $\pm$ 0.5	0.8 $\pm$ 0.8	0	0	1.7 $\pm$ 1.5	2.7 $\pm$ 0.7	22 $\pm$ 2.2	19 $\pm$ 3.5	0	0.2 $\pm$ 0.2	0	0.5 $\pm$ 0.3
	14/1/03	115 $\pm$ 18	0	0.3 $\pm$ 0.3	2.0 $\pm$ 0.7	1.5 $\pm$ 1.5	0.2 $\pm$ 0.2	0	0	0.7 $\pm$ 0.3	3 $\pm$ 0.6	18 $\pm$ 3.6	15 $\pm$ 6.9	0	0	0.3 $\pm$ 0.3	0.3 $\pm$ 0.2
	28/1/03	108 $\pm$ 7	0	0	0.2 $\pm$ 0.2	0	0	0	0	0.2 $\pm$ 0.2	1.8 $\pm$ 0.6	14 $\pm$ 2	25 $\pm$ 9.2	0	0	0	0
	11/2/03	195 $\pm$ 30	0	0	0.2 $\pm$ 0.2	0.8 $\pm$ 0.7	0	0	0	0.2 $\pm$ 0.2	3.3 $\pm$ 0.9	11 $\pm$ 2.6	29 $\pm$ 8.3	0	0.2 $\pm$ 0.2	0	0
Aut	25/2/03	164 $\pm$ 34	0	0	0.3 $\pm$ 0.3	2 $\pm$ 1.6	0.5 $\pm$ 0.3	0	0	1.8 $\pm$ 1.8	3.7 $\pm$ 0.9	12 $\pm$ 2.3	34 $\pm$ 12	0	0	0	0
	25/3/03	175 $\pm$ 45	25 $\pm$ 17	0	0	0.3 $\pm$ 0.3	0.8 $\pm$ 0.7	0	0	0.2 $\pm$ 0.2	8.7 $\pm$ 1.7	31 $\pm$ 5.6	43 $\pm$ 13	0	0	0	0
	22/4/03	110 $\pm$ 12	19 $\pm$ 5.2	0	0	0	0.2 $\pm$ 0.2	0	0	0.2 $\pm$ 0.2	4.2 $\pm$ 1.9	11 $\pm$ 3.2	25 $\pm$ 5.3	0	0	0	0
	25/5/03	236 $\pm$ 33	77 $\pm$ 23	6.7 $\pm$ 4.6	0	0	0.3 $\pm$ 0.2	0	0	2.8 $\pm$ 2.2	2.5 $\pm$ 0.8	7.5 $\pm$ 2	17 $\pm$ 5.4	0	0	0	0
W	24/6/03	15 $\pm$ 0	38 $\pm$ 13	6.1 $\pm$ 3.4	0	0	0.2 $\pm$ 0.2	0	0	4 $\pm$ 2.5	3 $\pm$ 1.1	12 $\pm$ 1.6	3.7 $\pm$ 1	0	0	0	0
	23/7/03	15 $\pm$ 0	25 $\pm$ 7.5	9.5 $\pm$ 1.3	1 $\pm$ 1	0	0.3 $\pm$ 0.3	0	0.5 $\pm$ 0.2	114 $\pm$ 56	6.3 $\pm$ 1.7	6.7 $\pm$ 2.2	7.3 $\pm$ 2.4	0	0	0	0
	27/8/03	15 $\pm$ 0	13 $\pm$ 2.8	17 $\pm$ 4.1	1.2 $\pm$ 0.8	0	0	0	1.7 $\pm$ 1.3	40 $\pm$ 11	2.3 $\pm$ 1.6	7.2 $\pm$ 1.5	4.5 $\pm$ 1.1	0	0	0	0
	21/9/03	15 $\pm$ 0	7 $\pm$ 2.9	11 $\pm$ 2.5	2 $\pm$ 0.7	0.5 $\pm$ 0.5	0.2 $\pm$ 0.2	0	0.2 $\pm$ 0.2	30 $\pm$ 14	8 $\pm$ 2	37 $\pm$ 9.7	9.8 $\pm$ 3.9	0	0	0	0
Sp	13/10/03	15 $\pm$ 0	0.2 $\pm$ 0.2	8.3 $\pm$ 3.7	5.2 $\pm$ 1.8	0	0	0	0.2 $\pm$ 0.2	6.5 $\pm$ 3.7	1.3 $\pm$ 0.3	31 $\pm$ 7.8	7.2 $\pm$ 2.1	0	0	0	0
	4/11/03	43 $\pm$ 7	0	1.3 $\pm$ 1	2.3 $\pm$ 0.8	0	0.2 $\pm$ 0.2	0	0	0.3 $\pm$ 0.3	1.5 $\pm$ 1	19 $\pm$ 6	6.2 $\pm$ 2.5	0	0	0	0
	25/11/03	91 $\pm$ 8	0	1.8 $\pm$ 1.1	2.7 $\pm$ 1.9	1.2 $\pm$ 0.7	0	0.5 $\pm$ 0.5	0	6.2 $\pm$ 2.7	2 $\pm$ 0.6	18 $\pm$ 6.1	3.3 $\pm$ 1.1	0	0	0	0
	15/12/03	187 $\pm$ 28	0	0	0.8 $\pm$ 0.5	0.8 $\pm$ 0.5	0	6.2 $\pm$ 5.8	0	0	2.3 $\pm$ 1	18 $\pm$ 3.7	4.3 $\pm$ 0.8	0	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0
Sum	6/1/04	196 $\pm$ 15	0	0	0	1 $\pm$ 0.7	0.2 $\pm$ 0.2	1.7 $\pm$ 1.7	0	0.7 $\pm$ 0.7	2.7 $\pm$ 0.8	10 $\pm$ 3.3	17 $\pm$ 4.6	0	0.7 $\pm$ 0.2	0.2 $\pm$ 0.2	0
	27/1/04	292 $\pm$ 62	0	0	0	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0	0.2 $\pm$ 0.2	3.5 $\pm$ 1.1	17 $\pm$ 4.9	23 $\pm$ 5.2	0	0	0	0
	18/2/04	206 $\pm$ 39	1.7 $\pm$ 1.7	0	0	0	0.2 $\pm$ 0.2	2.2 $\pm$ 2.2	0	0.7 $\pm$ 0.5	5.3 $\pm$ 1.3	14 $\pm$ 2.6	34 $\pm$ 19	0	0	0	0.2 $\pm$ 0.2
	11/3/04	201 $\pm$ 44	57 $\pm$ 36	0	0	0	0	0	0	0	3.2 $\pm$ 0.9	8.8 $\pm$ 2.9	25 $\pm$ 5.4	0	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.3 $\pm$ 0.2
Aut	5/4/04	129 $\pm$ 15	62 $\pm$ 38	9.7 $\pm$ 6.2	0	0	0	0	0	4 $\pm$ 3.8	4.8 $\pm$ 1.1	7 $\pm$ 1.5	14 $\pm$ 5.8	0	0	0.2 $\pm$ 0.2	1.3 $\pm$ 0.4
	10/5/04	206 $\pm$ 45	229 $\pm$ 82	39 $\pm$ 24.8	15 $\pm$ 15	0	0	0	0.3 $\pm$ 0.2	110 $\pm$ 64	20 $\pm$ 9.1	25 $\pm$ 15	35 $\pm$ 19	0	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	1.2 $\pm$ 0.7
W	21/7/04	160 $\pm$ 36	231 $\pm$ 81	51.7 $\pm$ 16	5.8 $\pm$ 5.8	0	0.5 $\pm$ 0.3	0	1.2 $\pm$ 0.6	54 $\pm$ 21	7 $\pm$ 1.5	7 $\pm$ 2	18 $\pm$ 8.2	0	0	0.2 $\pm$ 0.2	0.8 $\pm$ 0.5

Codes.

1: VG = Vegetative growth (cm), B = Buds, F = Flowers, GP = Green pods, BP = Black pods.

2: Ss = *S. staphylinus* adult (A) and juvenile (J) (Thripidae); Osp = *Odontothripiella* sp. adult (A) and juvenile (J) (Thripidae); Hv = *Haplothrips victoriensis* adult (A) and juvenile (J) (Phlaeothripidae); Phyto = Mites (Acari) in the family Phytoseiidae; Anys = Mites (Acari) in the family Anystidae; Ery = Mites (Acari) in the family Erythraeidae; Chey = Mites (Acari) in the family Cheyletidae, Michy = Microhymenoptera.

*Odontothripiella* sp. (Thysanoptera: Thripidae) adults were found on gorse in winter and spring (2003), then in autumn and winter (2004). Adult *Odontothripiella* sp numbers were significantly correlated with the number of flowers ( $r = 0.30$ ,  $p < 0.01$ ). However, the juveniles were present almost all year with their numbers most tightly correlated with the number of flowers ( $r = 0.49$ ,  $p < 0.00001$ ). Although found on all sampling dates, *Odontothripiella* sp. was the most abundant arthropod presented on Table 6.2 on only four sampling dates. These occurred in winter 2003 and autumn/winter 2004, these dates coincided with gorse flowering (Fig. 6.2d).

Both adults and juveniles of the omnivorous *Haplothrips victoriensis* (Bagnall) (Thysanoptera: Phlaeothripidae) were present on gorse on all sampling dates (Fig. 6.2e). They were the most abundant arthropods presented on Table 6.2 on 12 sampling dates, which occurred during spring and summer. Adult *H. victoriensis* numbers were significantly correlated with the numbers of buds ( $r = 0.43$ ,  $p < 0.00001$ ) flowers ( $r = 0.61$ ,  $p < 0.00001$ ) and especially green pods ( $r = 0.69$ ,  $p < 0.00001$ ) and were weakly correlated with the numbers of juvenile *Odontothripiella* sp. ( $r = 0.20$ ,  $p < 0.05$ ). Juvenile *H. victoriensis* numbers significantly correlated with the numbers of green pods ( $r = 0.49$ ,  $p < 0.00001$ ).

Mites in the family Phytoseiidae were present on all sampling dates at similar levels of abundance to *H. victoriensis* (Fig 6.2f). Phytoseiid mites were the most abundant arthropods presented on Table 6.2 on 11 sampling dates, which occurred mainly during autumn and summer with their numbers significantly correlated with the number of adult *H. victoriensis* ( $r = 0.43$ ,  $p < 0.00001$ ).

Microhymenopterans and mites (Acari) in the families Anystidae, Erythraeidae, Cheyletidae were present on gorse sporadically throughout the study.

Microhymenopterans were found in summer 2003 and summer, autumn and winter 2004 with their numbers significantly correlated with the number of adult *H. victoriensis* ( $r = 0.38$ ,  $p < 0.0001$ ). Anystid mites were only found on one sampling date in summer 2002. However, these mites are very mobile and one was also observed crawling on the outside of the Tullgren funnel apparatus in summer 2003. Erythraeid mites were found on gorse in summer 2002 and summer and autumn 2003. Finally, cheyletid mites were found on gorse in summer 2002 and summer, autumn and winter 2003.

## 6.4 Discussion

The interaction between plants, phytophagous arthropods and generalist predators or omnivores is highly complex and therefore difficult to predict. However, these interactions could affect the population dynamics of phytophagous arthropods used as weed biological control agents. Both individual species and groups of generalist predators have been shown to reduce both the numbers of phytophagous arthropods and the resulting plant damage (Symondson *et al.*, 2002). Similarly, omnivores that include arthropods as part of their diet can contribute to the stability of phytophagous arthropod populations (Coll and Guershon, 2002). In fact, there is evidence to suggest that local generalist predators can result in the extinction of certain introduced weed biological control agents in the country of release (Ireson *et al.*, 2002).

The genus *Odontothripiella* contains 18 flower feeding species endemic to Australia (Pitkin, 1972). Species of *Odontothripiella* are generally host specific and univoltine in the flowers of native legumes. The most widely distributed member of this genus is *O. australis* (Bagnall) (Pitkin, 1972). This species is widespread across southern Australia and polyphagous on native and introduced legumes (Mound and Gillespie, 1998), including lupins (*Lupinus* spp.) and gorse (L.A. Mound, personal communication). It is therefore likely that the *Odontothripiella* sp. collected in this study is *O. australis*, but due to the lack of males in the specimens provided for identification, the species identity could not be confirmed (L.A. Mound, personal communication). Indeed further samples taken of this species are yet to find a male specimen among them (J.E. Ireson, unpubl. data). Species of *Odontothripiella* are typical flower feeding Thripidae (Pitkin, 1972) and indeed this was the case on gorse, with its numbers highly correlated with gorse flowering. However, as these two thrips

species are both members of the same family (Thripidae), it is also possible that predators of *Odontothripella* sp. may also feed on *S. staphylinus*. The presence of this species all year and especially abundant in winter would enable thrips predators to also remain on gorse all year round. Furthermore, if predators of *Odontothripella* sp. inhabit gorse in large numbers when *S. staphylinus* is introduced, then it may be difficult to establish viable populations.

Mites in the families Anystidae, Erythraeidae and Cheyletidae have been reported as predators of species of thrips (Sabelis and Van Rijn, 1997). These groups were not consistently present on gorse in this study, although due to their high degree of mobility, it is suspected that the presence of the Anystidae was underestimated. Therefore, it is conceivable they were present more often than the results indicate. Mites in the family Phytoseiidae are highly effective specialist and generalist predators of many arthropod species (McMurtry and Croft, 1997). Many species of plant inhabiting phytoseiid mites feed on members of the family Thripidae (Sabelis and Van Rijn, 1997).

The genus *Haplothrips* contains some species that are probably purely phytophagous, some that may be purely predatory and others that are omnivorous (Mound and Gillespie, 1998). *H. victoriensis* Bagnall (Thysanoptera: Phlaeothripidae), was found to be predatory on *T. urticae* Koch (Acari: Tetranychidae) eggs and phytophagous on flowers of seed lucerne, *Medicago sativa* L. (Fabaceae) crops in South Australia (Bailey and Caon, 1986). It is also suspected to be a predator of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Goodwin and Steiner, 1996). When confined in a glass jar, second instar larvae of *H. victoriensis* will feed on second instar larvae of *S. staphylinus* (J.T. Davies and J.E. Ireson, personal

observation). Its strongly significant correlations with the stages associated with gorse flowering and weaker though significant correlation with juvenile *Odontothripiella* sp. numbers suggest that this species is strongly associated with gorse flowering and possibly acting as an omnivore.

As *H. victoriensis* and phytoseiid mites were both abundant on all sampling dates in this study, it is conceivable that they could be having a negative impact on populations of *S. staphylinus*, particularly in the earlier stages of population development following an initial release. Predation could therefore be a contributing factor to low population densities and the slow spread of *S. staphylinus* that was recorded in this study as well as at other release sites around the state (Ireson *et al.*, 2006). The low numbers of *S. staphylinus* seen in this study reduced the likelihood of finding significant relationship to potential predators. Further studies, such as a natural enemy exclusion experiment, would be required to test this hypothesis.

## **Chapter 7. The toxicity of commonly used herbicides and adjuvants used in gorse control on gorse thrips, *Sericothrips staphylinus*.**

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### **Abstract**

Two chemical residue bioassays were conducted on *S. staphylinus*, a biological control agent of gorse to determine the toxicity of four herbicides (triclopyr, triclopyr/picloram, metsulfuron-methyl and glyphosate) and two adjuvants (modified polydimethylsiloxane and soyal phospholipids/propionic acid). In the first bioassay, single ingredients at recommended field rates for gorse control were used. In the second bioassay, triclopyr was omitted, the remaining single products were tested and herbicide/adjuvant combinations were also tested. Triclopyr/picloram was found to be consistently ranked the most toxic chemical tested for both adults and larva II in both bioassays. The toxicity of the remaining treatments varied depending on the bioassay and insect stage tested. However, toxicity significantly higher than the control was also recorded for glyphosate, triclopyr and modified polydimethylsiloxane. The integration of chemical and biological control within an integrated management strategy for gorse is discussed.

## 7.1 Introduction

Gorse, *Ulex europaeus* L. (Fabaceae) is a Weed of National Significance in Australia (Thorp, 1999) and has a serious impact on agricultural land and environmentally significant regions in South Eastern Australia, predominantly Victoria and Tasmania. As part of an integrated management strategy, a suite of host-specific biological control agents are currently being investigated for gorse control in Australia (Ireson *et al.*, 2004). The gorse thrips, *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae), was introduced to Australia in January 2001 and has since established but is spreading slowly (Ireson, *et al.*, 2006).

Partial control is the most common result of weed biological control programs (McFayden, 1998). Therefore, other control methods, including chemical control with herbicides will remain an integral component of an integrated weed management strategy. The management of gorse infestations using biological control alone will not be possible in the short term (Ireson, *et al.*, 2006), therefore the use of herbicides will continue into the foreseeable future.

Herbicides that are registered in Australia and recommended to use against gorse include triclopyr (eg. Garlon<sup>®</sup>), triclopyr/picloram (eg. Grazon<sup>®</sup>), metsulfuron-methyl (eg. Brush off<sup>®</sup>), glyphosate (eg. Round up<sup>®</sup>) and ammonium thiocyanate (eg. Amitrole T<sup>®</sup>) (Anonymous, 1997; Anonymous, 2005). Adjuvants (surfactants or penetrants) are often combined with herbicides to improve their effectiveness. Adjuvants recommended to be used in conjunction with some of the above mentioned herbicides include the penetrant modified polydimethylsiloxane (MP) (eg. Pulse penetrant<sup>®</sup>), and the surfactant soyal phospholipids plus propionic acid (SPPA) (eg. LI700<sup>®</sup>).



Parsons and Cuthbertson (2001) summarise the properties of the above-mentioned herbicides, which are grouped into three specific mode of action categories.

Glyphosate (mode of action grouping M) [*N*-(phosphonomethyl)glycine] is a systemic, organophosphorus, leaf absorbed compound that inhibits enzyme activity at growing points, including roots. Metsulfuron methyl (mode of action grouping B) [2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoic acid] is a sulphonyl urea compound which is rapidly absorbed by leaves and translocated throughout the plant, affecting cell division at growing points. Picloram (mode of action grouping I) [4-amino-3,5,6-trichloropyridine-2-carboxylic acid] is a substituted pyridine compound, readily absorbed by stems leaves and roots, which moves rapidly through the plant interfering with cell elongation and other processes. Triclopyr (mode of action grouping I) [3,5,6-trichloro-2-pyridyloxyacetic acid] is a leaf absorbed, auxin-like compound which is well translocated and interferes with cell division. Adjuvants are chemicals that are used in conjunction with herbicides to increase their efficacy. MP is a penetrant that has the ability to open up the plant wax layer on the cuticle surface and enable the herbicide to get in more effectively, whereas SPPA is a low rate surfactant that breaks the surface tension therefore preventing beading of droplets on the leaf surface.

The effect of these chemicals on adult and juvenile *S. staphylinus* is unknown. Insects may be affected by herbicides or the additives used with them at least during part of their life cycle. Interactions between biocontrol agent and a herbicide can be unfavourable, favourable or neutral (Messersmith and Adkins, 1995). Herbicides can have a negative impact on insect populations by causing direct mortality to certain insect stages, affecting oviposition, having anti-feedant properties or indirectly by destroying the food supply (Messersmith and Adkins, 1995). Evaluating the

interaction between herbicides and biocontrol agents at an early stage of insect establishment is recommended by Messersmith and Adkins (1995) to improve weed biological control.

Bioassays are used to quantify the effect a given chemical has on an insect. Several bioassay methods have been developed to determine the impact of insecticides on insects (Busvine, 1980), especially regarding the measurement of pesticide resistance. Topical applications or residue tests are common methods of conducting bioassays on species of thrips (Thysanoptera) to determine the direct toxic effect of a given chemical (Lewis, 1997 a).

Integrated Weed Management strategies combining multiple control methods are becoming increasingly important as single control strategies are rarely completely effective. Chemical and biological control methods are both important strategies within an Integrated Weed Management strategy and therefore the compatibility of these two methods should be evaluated to allow improved decision making. Apart from the desired toxicity to the target weed, herbicides and adjuvants can be toxic to arthropods.

A slide dip bioassay was conducted in New Zealand on the gorse spider mite, *Tetranychus lintearius* Dufour (Acari: Tetranychidae) using herbicides and adjuvants that are commonly used for gorse control in New Zealand (Searle *et al.*, 1990). In this study it was found that certain herbicides (especially triclopyr, triclopyr/picloram and glyphosate) and surfactants can be toxic to adult female *T. lintearius*, when tested alone or in combinations of herbicide and adjuvant.

It is possible that some herbicides and adjuvants commonly used to control gorse in Tasmania will have an antagonistic effect on *S. staphylinus*, thus interfering with

biological control. Identification of these chemicals will allow better decision making within an integrated weed management strategy. Therefore, the aim of this study was to conduct laboratory tests on the toxicity of commonly used herbicides and adjuvants for gorse control on adult and juvenile *S. staphylinus*.

## 7.2 Materials and Methods

### 7.2.1 Collection and rearing of *S. staphylinus*

*S. staphylinus* of English origin (via New Zealand), that was first released in Australia in 2001 (Ireson *et al.*, 2004), was used in both bioassays. Rearing was conducted in a temperature-controlled glasshouse maintained between 20 and 25°C with a 16-h photoperiod. The *S. staphylinus* used in both bioassays originated from a large, continuous culture maintained in the glasshouse on numerous potted gorse plants.

Bioassays were conducted on juvenile and adult *S. staphylinus*. Second stage larvae (larva II) were the juvenile stage chosen for the bioassays as they are larger and more mobile than first stage larvae and therefore more likely to come into contact with chemical residues on gorse plants. Adult females were chosen for the same reason (Lewis, 1997 b). As *S. staphylinus* cannot be sexed reliably without examining mounted specimens, probable adult females were selected for use in the bioassay by choosing thrips with abdomens wider than their thorax (Hill *et al.*, 2001).

Larva II were obtained for the bioassays directly from the glasshouse culture. To obtain *S. staphylinus* adults of approximately the same age for each bioassay, three potted gorse plants were each infested with approximately 200 larva II collected from the glasshouse culture. After 17 days, the first adults were noted. Fourteen days after this time, when all *S. staphylinus* in the culture were adults, the bioassays were conducted.

### 7.2.2 Chemicals and combinations

The chemicals and concentrations used were those recommended for gorse control (Anonymous, 1997; Anonymous, 2005). Two separate bioassays were conducted to

test the effect of herbicides and adjuvants on the mortality of *S. staphylinus* (Table 7.1). In bioassay 1, single ingredients at recommended field rates for gorse control were used and herbicide/adjuvant combinations were tested in bioassay 2. In this latter bioassay, triclopyr was omitted as it was found to have similar toxicity to triclopyr/picloram, but the remaining three herbicides were tested at identical rates individually and in combination with the two adjuvants.

**Table 7.1.** Chemical (4 herbicides and 2 adjuvants) combinations and rates used in the glass jar residue bioassay of the *Sericothrips staphylinus*.

<i>Chemical or chemical combination</i>	<i>Rate</i>	<i>Bioassay 1</i>	<i>Bioassay 2</i>
Distilled water (control)	n/a	✓	✓
Triclopyr (300 g/L)/picloram (100 g/L)	5 mL/L	✓	✓
Triclopyr (600 g/L)	3.4 mL/L	✓	×
Metsulfuron-methyl (600 g/kg)	1.5 g/L	✓	✓
Glyphosate (360 g/L)	10 mL/L	✓	✓
<sup>1</sup> MP (adjuvant)	2 mL/L	✓	✓
<sup>2</sup> SPPA (adjuvant)	1 mL/L	✓	✓
Triclopyr (300 g/L)/picloram (100 g/L) + MP	*	×	✓
Triclopyr (300 g/L)/picloram (100 g/L) + SPPA	*	×	✓
Metsulfuron-methyl (600 g/kg) + MP	*	×	✓
Metsulfuron-methyl (600 g/kg) + SPPA	*	×	✓
Glyphosate (360 g/L) + MP	*	×	✓
Glyphosate (360 g/L) + SPPA	*	×	✓

\*As above for individual chemicals

<sup>1</sup>MP = Modified Polydimethylsiloxane (1000g/L) (Pulse Penetrant®)

<sup>2</sup>SPPA = Soyol Phospholipids (350g/L), Propionic Acid (350 g/L) (LI700®)

### 7.2.3 Bioassay method

The bioassays were conducted using a chemical residue test inside glass tubes similar to the methods described by Robinson and Hoffman (2000). Glass tubes, 10 cm in length and 2.5 cm in diameter, were prepared by thoroughly washing in warm soapy

water then draining and triple rinsing in distilled water, with time allowed for thorough draining between each rinse. A 5ml preparation of each chemical and chemical combination (Table 7.1) was dispensed into each glass tube using a pipette. Each tube was then tilted almost to a horizontal position and gently swirled so that the chemical coated the entire inside of each tube. The chemical was then poured out of the tubes, these were left to drain in an operating fume hood for approximately three hours until all of the liquid had dried leaving a dried residue of each chemical in the tubes.

As *S. staphylinus* were determined to be prone to dessication in pilot trials (J.T Davies, unpublished data), a 1 cm piece of freshly cut gorse spine was added to each tube before the addition of *S. staphylinus* to allow a source of food and moisture.

*S. staphylinus* were beaten from potted gorse plants from the glasshouse culture onto a white tray and aspirated directly into prepared tubes using a modified electrical pooter. An average  $\pm$  SE of  $1.8 \pm 0.08$  (range 1-4) larva II and  $1.9 \pm 0.06$  (range 1-4) adult *S. staphylinus* were pooted into each tube for bioassay 1 and an average  $\pm$  SE of  $1.9 \pm 0.06$  larva II (range 1-4) and  $2.1 \pm 0.06$  (range 1-4) adults for bioassay 2. Ten replicate tubes were used for each chemical or chemical combination in both bioassays. Following thrips introduction, each tube was covered with Parafilm<sup>®</sup>. Prepared bioassay tubes containing *S. staphylinus* were stored in a temperature controlled cabinet at 22.5°C for 24 hours before mortality assessment.

Mortality was assessed for both adult and larva II *S. staphylinus* by gently touching the thorax region with a fine sable hair brush, if leg movement was detected within 5 seconds they were considered alive, if no leg movement was detected within 5 seconds, they were considered dead.

#### 7.2.4 Data analysis

Statistical tests were performed using SYSTAT 10th edition. In both bioassays, percentage mortalities due to each chemical or chemical combination were calculated per tube for both larva II and adult *S. staphylinus*, this data was arcsine square root transformed. Initially, analyses of variance were performed in both bioassays with stage (larva II or adults) and chemical treatment as factors.

As there was no significant difference between the mortality rate of larva II or adult *S. staphylinus* in bioassay 1 (see results), an analysis of variance was performed between each chemical treatment/stage combination. A Tukey test was then performed to separate means. However, in bioassay 2 there was a significant difference between the mortality rate of larva II and adult *S. staphylinus* (see results). In this case the analysis of variance was performed separately for larva II and adult *S. staphylinus* to test for differences between treatments of chemicals and chemical combination. Tukey tests were performed separately for larva II and adult *S. staphylinus* mortality to separate means.

## 7.3 Results

### 7.3.1 Bioassay 1

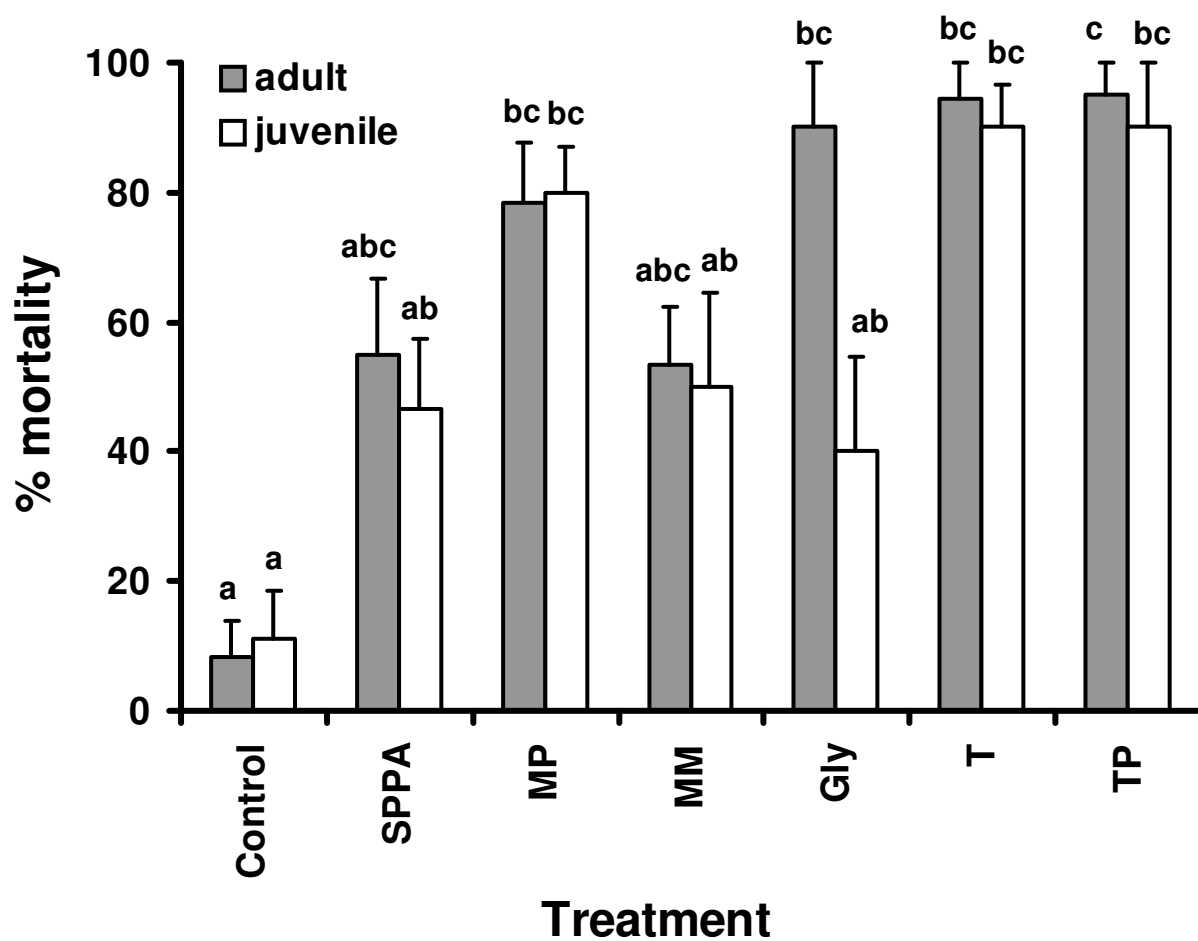
There was a significant difference in *S. staphylinus* mortality between the chemical treatments ( $F_{6,126} = 17.2$ ,  $P < 0.001$ ). There was no significant difference between the mortality of larva II and adult *S. staphylinus* ( $F_{1,126} = 3.2$ ,  $P = 0.074$ ) and there was also no significant interaction between chemical treatment and insect stage (larva II and adult) ( $F_{6,126} = 1.8$ ,  $P = 0.1$ ).

The majority of all chemical treatments caused higher mortality levels than the control (Larva II mortality - 8.3%, Adult mortality - 11.1%) (Fig. 7.1). Of the chemical treatments, glyphosate (on larva II), SPPA (on adults and larva II) and metsulfuron-methyl (on adults and larva II) had the lowest mortality levels (between 40 and 55%), which were not significantly higher than the control. However, MP (on adults and larva II), glyphosate (on adults), triclopyr (on adults and larva II) and triclopyr/picloram (on adults and larva II) all had significantly higher mortality levels than the control (between 78% and 95%).

### 7.3.2 Bioassay 2

As in bioassay 1, there was a significant difference in *S. staphylinus* mortality between the chemical treatments ( $F_{11,216} = 6.1$ ,  $P < 0.001$ ). However, in contrast to bioassay 1, there was also a significant difference between the mortality of larva II and adult *S. staphylinus* ( $F_{1,216} = 36.2$ ,  $P < 0.001$ ). Across all treatments, the average mortality for larva II *S. staphylinus* (59%) was higher than the mortality of adults (33%). There was no significant interaction between chemical treatment and insect stage (larva II and adult) ( $F_{11,216} = 1.0$ ,  $P = 0.4$ ).

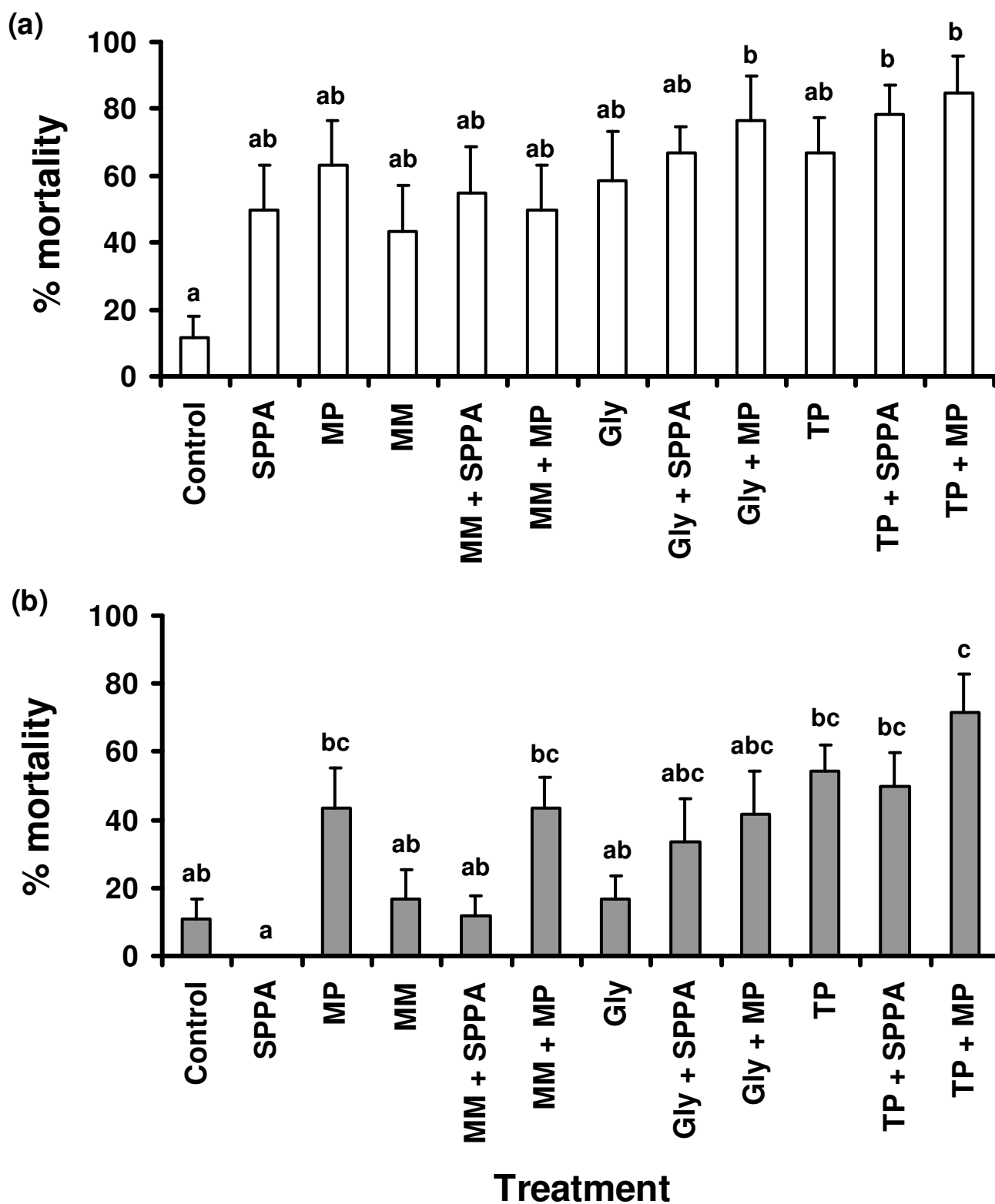




**Figure 7.1.** Mean  $\pm$  SE percentage mortality of adult and juvenile (larva II) *S. staphylinus* exposed to field rates of herbicides and adjuvants commonly applied to gorse, Bioassay 1. N = 10 in all cases; means with the same letters are not significantly different ( $P < 0.05$ ; Tukey Test). Key to codes: Control = distilled water, SPPA = Soyal Phospholipids plus Propionic Acid, MP = Modified Polydimethylsiloxane, MM = Metsulfuron Methyl, Gly = Glyphosate, T = Triclopyr, TP = Triclopyr/Picloram.

When juvenile (larva II) *S. staphylinus* were assessed independently, the only treatments to cause a significantly higher mortality than the control (11.7%) were the chemical combinations glyphosate plus MP (76.7%), triclopyr/picloram plus SPPA (78.3%) and triclopyr/picloram plus MP (85%) (Fig. 7.2a). When adult *S. staphylinus* were assessed independently, the SPPA treatment (0%) and the control (10.8%) both had low levels of mortality. The only treatment to cause a significantly higher mortality than the control was the chemical combination triclopyr/picloram plus MP (Fig. 7.2b). This chemical combination caused the highest mortality of all treatments (71.7%).

When the herbicides and adjuvants were used in combination, the adjuvants appeared to slightly, although not significantly, increase the mortality caused by the herbicides, (Fig. 7.2a and b).



**Figure 7.2.** Mean  $\pm$  SE percentage mortality of (a) juvenile (larva II) (b) adult *S. staphylinus* exposed to field rates of herbicides commonly applied to gorse, Bioassay 2. N = 10 in all cases; means with the same letters are not significantly different ( $P < 0.05$ ; Tukey Test). Key to codes: Control = distilled water, SPPA = Soyab Phospholipids plus Propionic Acid, MP = Modified Polydimethylsiloxane, MM = Metsulfuron Methyl, Gly = Glyphosate, TP = Triclopyr/Picloram.

### 7.3.3 Comparison of bioassays 1 and 2

When treatment combinations in bioassay 2 were omitted and identical treatments were compared between both bioassays, there are clear differences in the results. In bioassay 1, the average mortality for adults (63%) is slightly higher than the average mortality for larva II (53%). However, in bioassay 2 the average mortality for adults (23.6%) is less than half the average mortality of the larva II (48.9%). Furthermore, the average adult mortality in bioassay 1 is more than double that of bioassay 2, whereas the average mortality for juveniles is similar between both bioassays. When individual chemicals are compared, there are much bigger differences between the bioassays in adult mortality than for larva II mortality (% bioassays 1 - % bioassay 2: *Adults* control = -2.5, MM = 36.6, Gly = 73.3, TP = 40.8, SPPA = 55.0, MP = 35, adult mean = 39.7; *Larva II* control = - 0.6, MM = 6.7, Gly = -18.3, TP = 23.3, SPPA = -3.3, MP = 16.7, larva II mean = 4.1).

When the same single ingredient treatments are ranked according to mortality, a trend is evident. Triclopyr/picloram consistently resulted in the highest mortality for both adults and larva II in both bioassays. MP resulted in the second highest mortality for juveniles in both bioassays and adults in bioassay 2. For adults in bioassay 1, MP was the third most toxic treatment. The ranking according to mortality of the remaining single ingredient treatments was varied.

## 7.4 Discussion

Triclopyr/picloram was consistently ranked the most toxic herbicide tested in this study, both to *S. staphylinus* adults and larva II in both bioassays. Of the two adjuvants, MP was toxic and both appeared to elevate the toxicity, though not significantly, of the herbicides when they were tested in combination. The combination of triclopyr/picloram and the adjuvant MP was ranked the most toxic chemical combination against adult *S. staphylinus* in bioassay 2. Of the herbicides tested in this trial, triclopyr/picloram is also considered to be the most effective at controlling gorse (Anonymous, 1997). This is an important factor in the development of an integrated management strategy for gorse. An application of this chemical under field conditions during periods of high *S. staphylinus* activity could result in a significant reduction of *S. staphylinus* populations and ultimately reduce the impact that the agent may otherwise have had on gorse.

Further studies on the compatibility of chemical and biological control using *S. staphylinus* need to focus on the impact of herbicide applications on populations of *S. staphylinus* inhabiting gorse in a field environment. It is currently recommended that the optimum time to apply triclopyr/picloram is when gorse is actively growing (Anonymous, 1997), which would occur from spring through to autumn in Tasmanian conditions depending on local conditions. As the activity of *S. staphylinus* juvenile stages peaks in late spring/early summer in Tasmania (see Chapter 6, Table 6.2 and J.E. Ireson, unpublished data), it is likely that herbicide applications in late autumn would have a lower impact on *S. staphylinus* populations than would earlier applications in spring and summer. However, late autumn applications could have an

impact on populations of over-wintering adults and impact recruitment in spring/summer.

Other than direct toxicity, herbicides may also interfere with an insect population indirectly, such as by destroying the food supply (Messersmith and Adkins, 1995). If the gorse plants die from a herbicide application, this may leave *S. staphylinus* without a food source and they may not survive when the gorse reinfests the area from its large and persistent seed bank. Maintenance of unsprayed gorse plants may help to maintain a population of *S. staphylinus* to allow reinfestation of re-emerging gorse. Also, the mortality of *S. staphylinus* may vary according to the timing of plant death. If a herbicide application is timed so that the plants die when mobile adults are present, such as in autumn, the mortality may be lower than if the herbicide was applied in late spring or early summer when lots of juveniles are present.

The results with larva II were more consistent across the two bioassays than the results with adults. Several factors may affect the result of bioassays (Busvine, 1980), which may explain the discrepancy with the adult results. Extrinsic factors such as temperature, humidity, food supply, illumination and timing of the tests was considered highly unlikely, as these factors were identical between the bioassays and little discrepancy with larva II mortality was reported. An intrinsic factor or combination of factors was therefore more likely.

Susceptibility to chemicals often decreases with increasing size (Busvine, 1980), and as this factor was not measured in either bioassay, this factor cannot be ruled out. Females are often less susceptible than males (Busvine, 1980), which may be related to their larger size. The sex of adult *S. staphylinus* used in both bioassays was determined as female (see materials and methods and Hill *et al.*, 2001). However, it is

possible that a difference in the ratio of female: male *S. staphylinus* per tube may have caused the discrepancy between the two bioassays.

Busvine (1980) also mentions there is often a period of high susceptibility to chemicals soon after adult emergence, which can be followed by a period of higher tolerance to chemicals then increased susceptibility again with advancing age. Adult *S. staphylinus* used in both bioassays were collected 14 days after the appearance of the first adults and were therefore considered to be of a similar age. Although the *S. staphylinus* used in the bioassays were reared in a temperature controlled glasshouse, considerable variation in temperature existed, particularly when outside temperature varied. It is therefore possible that the adults used in the bioassays were of a different physiological age which resulted in the adults of the first bioassay being more susceptible to the chemical treatments than the adults in the second bioassay.

Despite the reported discrepancy, this preliminary study has identified that herbicides and adjuvants can cause significant mortality to *S. staphylinus*. It is recommended that further studies be conducted on the effect of field herbicide applications on *S. staphylinus* populations and the integration of herbicides with biological control. These studies could investigate the timing of herbicide applications, dispersal of mobile stages from dying gorse plants and maintaining small patches of unsprayed gorse to allow reinfestation by *S. staphylinus* to re-established gorse.

## Chapter 8. General discussion

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### 8.1 Introduction

The primary aim of this study was to assess the individual impact of *Exapion ulicis* (gorse seed weevil), *Tetranychus lintearius* (gorse spider mite) and *Sericothrips staphylinus* (gorse thrips) on the growth and development of gorse. This chapter summarises the major findings of this study and discusses the future of biological control of gorse in Australia and its role within an integrated weed management strategy.

### 8.2 Impact of the gorse spider mite

In a field experiment conducted over two and a half years, *T. lintearius* caused a reduction in dry matter production of approximately 36% (see Chapter 2), which suggests that this species has the potential to be a useful agent. However, a threat to the effectiveness of this agent is predation by natural enemies including *Stethorus* spp. and the Chilean predatory mite, *Phytoseiulus persimilis*. An experiment showed *P. persimilis* will develop at a similar rate on a diet of *T. lintearius* as it would on the two-spotted mite and that its generation time relative to its host is fast (see Chapter 3). This confirms the ability of this species to have a negative impact on *T. lintearius* populations. As larger *T. lintearius* populations will presumably increase the damage to gorse, it is highly likely that *P. persimilis* will reduce the effectiveness of *T. lintearius* as a biological control agent of gorse.

*T. lintearius* attacks foliage and reduces growth on mature plants. However, the damage sustained in this study was recorded in the early stages of *T. lintearius*



introduction into the area and it is likely that predation will limit the impact of this agent resulting in low levels of patchy damage to gorse in the long term.

As *T. lintearius* populations are likely to fluctuate considerably over extended time periods, further research, such as predator and prey exclusion experiments, are needed to investigate the tri-trophic interactions between gorse, *T. lintearius* and its predators to determine the resulting long term impact of *T. lintearius* on gorse.

### **8.3 Impact of the gorse seed weevil**

*E. ulicis* was found to reduce seed production of gorse at two sites in Tasmania (see Chapter 4). Due to differences in the patterns of pod production between the sites, the percentage of mature seed damaged in black pods for the whole 20 months of sampling was 2.7 times higher at Stonehenge (45.5%) than at Lymington (16.7%).

Rees and Hill (2001) determined that to reduce seed production of gorse below replacement levels, a reduction in seed production in excess of 90% would be required. Although *E. ulicis* reduced seed production, the levels obtained in this study are not considered enough to have an impact on gorse populations. Therefore, an additional seed feeding biological control agent (or agents) would be required if seed feeding agents are to have any impact on gorse populations in Australia.

Synchronisation of *E. ulicis* larval activity with seed production of its host, and the resulting impact on gorse seed production, was variable across the two sites studied. This variability in the pod production patterns is probably due to climatic differences between the sites. Therefore, studies on the pod production patterns of gorse at a range of sites will enable a more detailed understanding of the impact of *E. ulicis* on gorse seed production.

#### 8.4 Impact of the gorse thrips

In a glasshouse experiment, *S. staphylinus*, ryegrass competition and simulated grazing individually reduced the growth of gorse seedlings. It was also demonstrated that *S. staphylinus* could reduce gorse seedling survival when used in conjunction with ryegrass competition and simulated grazing (see Chapter 5). If similar combinations of *S. staphylinus*, competition from pasture species, and grazing management also affect gorse seedling survival under field conditions, then the *S. staphylinus* could become an important component of an integrated management strategy, especially in the earlier stages of gorse establishment.

However, in a field environment, significant damage to gorse plants has not yet occurred five years after introduction into Australia (J. E. Ireson, pers.comm., 2006). As population build up in the field has been slow, further studies on the population ecology of this species are required.

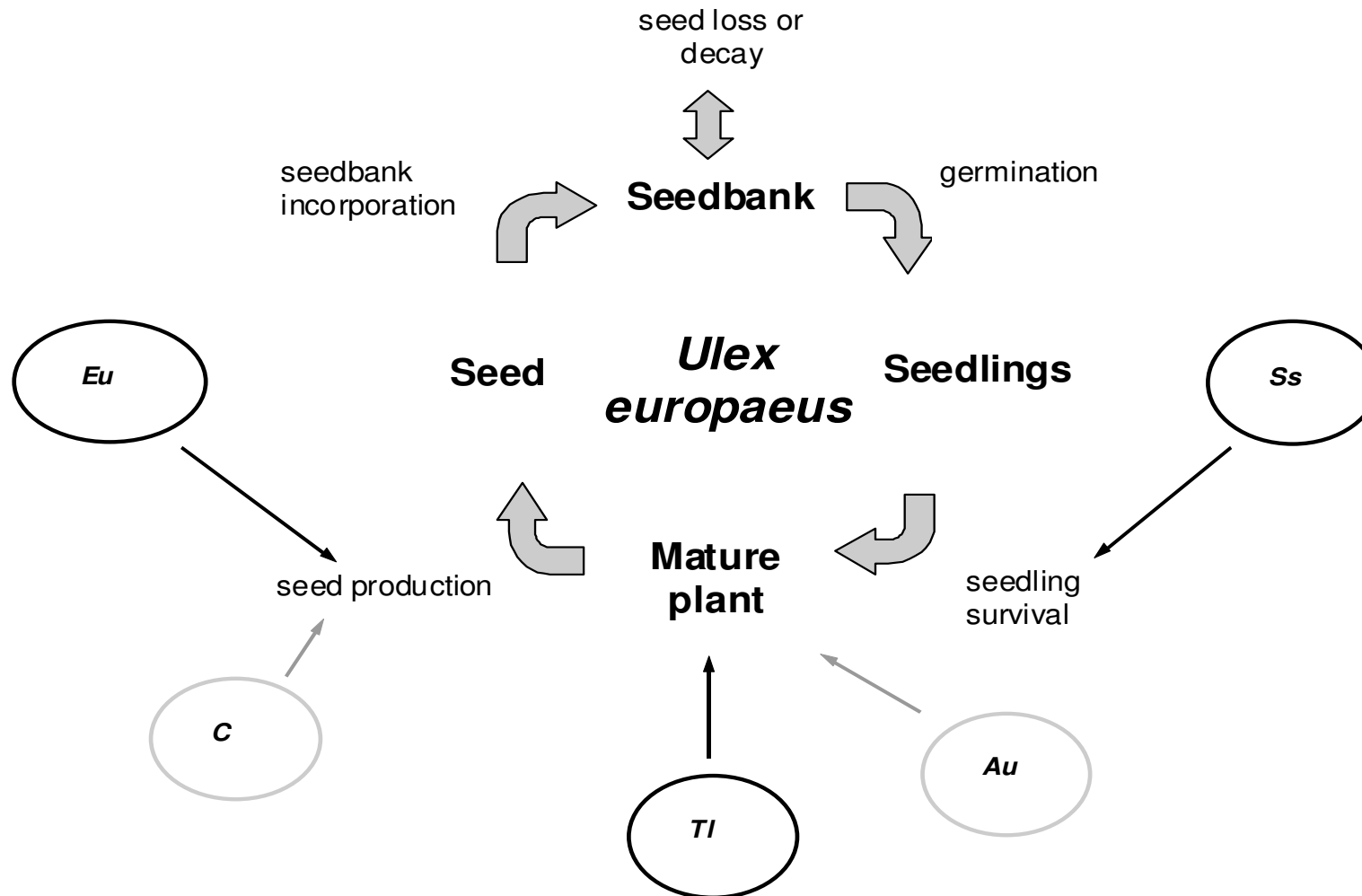
A field study was conducted to identify potential natural enemies of *S. staphylinus* within the arthropod fauna inhabiting gorse (see Chapter 6). The Phlaeothripid *Haplothrips victoriensis* and mites in the family Phytoseiidae were the most abundant predatory arthropods present on gorse throughout the study and are also reported to be natural enemies of other members of the family Thripidae. Further research, such as a predator exclusion experiment, is needed to determine the impact of these predators on *S. staphylinus* populations.

A preliminary bioassay was conducted to determine the toxicity of herbicides and adjuvants commonly used to control gorse on adult and juvenile *S. staphylinus* (see Chapter 7). The herbicides triclopyr/picloram, triclopyr and glyphosate were found to be toxic to *S. staphylinus*, as was the adjuvant modified polydimethylsiloxane. Further

research is required to determine the toxicity of these chemicals to *S. staphylinus* in a field situation. These studies could investigate the timing of herbicide applications, dispersal of mobile stages from dying gorse plants and maintaining small patches of unsprayed gorse to allow reinfestation by *S. staphylinus* to re-established gorse. However, an assessment of the impact of *S. staphylinus* in a field environment should be conducted before any further recommendations regarding the use of herbicides is made.

### **8.5 The role of biological control within an integrated management strategy for gorse in Australia**

The three agents investigated in this study all had a measurable impact on the performance of gorse and exert their impact at different stages of the gorse lifecycle (Fig. 8.1). *T. lintearius* attacks foliage and reduces growth on mature plants, *E. ulicis* reduces seed production and *S. staphylinus* possibly has an impact on younger plants. Although all three agents had an impact on gorse, a measurable impact on plant performance does not necessarily translate into an impact at the plant population level (Crawley, 1989). Earlier findings now coupled with those found within this thesis suggest that additional biological control agents will be required if biological control is to be considered an important long-term component of an integrated management strategy for gorse in Australia.



**Figure 8.1.** Schematic representation of the lifecycle of gorse (*U. europaeus*) displaying the points in the lifecycle where the biological control agents established (black ovals) and not established (grey ovals) in Australia have their greatest impact. Key: *Eu* = *Exapion ulicis*, *Cs* = *Cydia* sp., *Tl* = *Tetranychus lintearius*, *Au* = *Agonopterix ulicetella*, *Ss* = *Sericothrips staphylinus*.

A tortricid moth originally identified as *Cydia succedana* Denis and Schiffermüller (Lepidoptera: Tortricidae), that has since been identified as *Cydia ulicetana* Haworth (Q. Paynter, Landcare Research New Zealand, pers. comm., 2005) is now widely established in New Zealand. This species attacks gorse seed (Fig. 8.1) and is assumed by Hill and Gourlay (2002) to be bi-voltine attacking gorse seed produced in spring and autumn in New Zealand. However, its release into Australia is now unlikely (Ireson *et al.*, 2006) as there is evidence that this species uses other legume species as alternative hosts to gorse (Fowler *et al.*, 2004).

A survey was conducted in Western Europe (Portugal and Spain) in autumn 2003 with the aim of identifying potential biological control agents of gorse that are active in autumn (Sheppard, 2004). This survey was at the evolutionary centre of origin of the genus *Ulex* and targeted autumn active seed feeding insects. Low numbers of gorse seed were produced during this period and seed losses due to pod moths of the genus *Cydia* and seed weevils of the genus *Exapion* were minor (7 and 0.1% respectively). No other autumn active seed feeding insects were found. Surveys for root feeding and stem boring insects were conducted in spring and summer 2005 (Sheppard and Thomann, 2005). No insects were found in this study that showed any potential as biological control agents in the exotic range of gorse. Surveys for fungal pathogens are scheduled for 2006 (Ireson, *et al.*, 2006).

The gorse soft shoot moth, *Agonopterix ulicetella* (Stainton) (Lepidoptera: Oecophoridae), has recently been approved for release as a biological control agent for gorse in Australia and field releases are planned for Victoria and Tasmania in 2006 (Ireson *et al.*, 2006). This species has been released in New Zealand (Hill *et al.*, 1995), Hawaii (Markin *et al.*, 1996) and Chile (Norambuena *et al.*, 2004). Larvae of

*A. ulicetella* feed on the tips of gorse shoots and developing green spines in spring and early summer causing damage to foliage (Fig. 8.1).

If successful establishment of *A. ulicetella* occurs, the guild of biological control agents of gorse in Australia will consist of one seed feeder and three foliage feeding agents. The level of impact that this combination of agents will have on gorse in the long term will only be determined by future research.

The fundamental aim of an integrated weed management strategy is to reduce weed populations below economically damaging levels using multiple, compatible control techniques. The overall strategy must therefore have a significant impact on the population dynamics of the target weed to be successful. Biological control is just one technique used within an integrated management strategy. Other techniques that could be incorporated into an integrated management strategy for gorse include chemical control with appropriate herbicide applications and cultural control methods including burning, cultivation, grazing, manual removal and competition from pasture or other desirable plant species (King, *et al.*, 1996).

Hoffman (1990) states: 'Success in weed biological control can only be claimed when it is shown through suitable evaluation that the agents have caused a decrease in weed density'. Therefore according to this definition, a successful weed biological control program should have a significant impact on the population dynamics of its host.

However, determining such an impact of biological control on the population dynamics of a weed is a difficult prospect. It often takes a long period of time for an agent to establish and begin to exert an influence on the weed (McFayden, 1998) and experimental studies need to be conducted over a similarly lengthy time period. The impact of a particular agent may also vary from region to region (eg. Kelly and

McCallum, 1995) due to a host of environmental and ecological factors. Therefore, if a reduction in weed density isn't detected in one particular region, this doesn't necessarily hold true for all regions. Furthermore, if a particular herbivorous arthropod doesn't have an effect on plant population dynamics alone, it may have an incremental effect when combined with the effects of other herbivorous arthropods, resulting in substantial effects on plant population dynamics (Crawley, 1989).

In regard to having an impact on weed population dynamics, biological control should be considered a component of an integrated weed management strategy. If biological control contributes to a decline in a weed population as part of a total strategy, then biological control could be considered a useful component of the strategy even without directly causing the decline if used alone. Similar to the situation with individual biological control agents mentioned above, biological control (using single or multiple agents) may not have an effect on plant population dynamics. However, biological control may have an incremental effect when combined with the effects of other management strategies (such as pasture competition, grazing, burning and application of herbicides), which may result in substantial effects on plant population dynamics.

For example in this study, *S. staphylinus* used alone reduced the growth rate of gorse seedlings but did not cause any seedling mortality (see Chapter 5). However, when *S. staphylinus* was used in conjunction with ryegrass competition and grazing, mortality was increased to 93%. In this case a synergistic interaction was present where the combined impact is greater than the additive impact of each factor. Even if a synergistic interaction is not present in a field situation and the more common multiplicative interaction is found (where neither factor influences the other), then the

combined effect of biological control and other management strategies may still cause a reduction in weed abundance.

Interspecific competition from desirable plant species is recognized as an important factor contributing to the impact of biological control, especially in regard to invasive pasture weeds (Sheppard, 1996). However, the range of other management techniques in an integrated management program can also strongly influence the impact exerted by the weed biological control agent. An understanding of the factors that contribute to the impact of biological control agents can greatly improve integrated weed management decisions by identifying options, such as chemical or cultural methods that may enhance this impact (Farrell and Lonsdale, 1997).

Knowledge of the weed population dynamics provides a basis for the development of integrated weed management strategies as weaknesses can be identified that can then be targeted. In a study of gorse populations, simulation and analytical models were developed for gorse (Rees and Hill, 2001). The potential impact of seed feeding biological control agents on gorse abundance under several environmental and management scenarios were predicted in this study. The success of biological control was found to depend critically on the frequency and intensity of disturbance, whether disturbed sites became suitable for recruitment and the effects of disturbance on germination and seed mortality.

Previous studies have also examined the population dynamics and biological control of other weeds including scotch broom (*Cytisus scoparius*) (Rees and Paynter, 1997), ragwort (*Senecio jacobaea*) (McEvoy and Coombs, 1999) and prickly acacia (*Acacia nilotica*) (Kriticos *et al.*, 1999). These studies have also highlighted that recruitment



(ie. the entry of new individuals into a population through reproduction or migration) is a weakness in the weeds lifecycle that can be exploited.

An integrated management strategy for gorse that reduces seedling survival, prevents or substantially reduces subsequent recruitment and kills established plants will maximise the effect of biological control using seed feeding agents. When the survival of seedlings was 1%, it was predicted that a reduction in annual seed production by 90% resulted in gorse population decline (Rees and Hill, 2001).

A reduction of gorse seed production may be achievable in some regions by a combination of seed feeding agents (see Chapter 4 and Hill and Gourlay, 2002). However, this is only possible if seedling survival can be kept at low levels (below 1%) (Rees and Hill, 2001). Management strategies to reduce seedling survival could include combinations of herbicide applications, oversowing with suitable pasture species, grazing management (Rees and Hill, 2001) and biological control using *S. staphylinus*.

## **8.6 Conclusions**

This study has provided information on the impact of three biological control agents on gorse. As the impact of the agents was limited, it is recommended that for the short to medium term, reliance should be placed on control methods other than biological control for managing gorse populations. In the longer term, it is recommended that research into finding additional biological control agents for gorse be continued.

Initially, biological control agents should be released in gorse infestations where access is difficult and the opportunity for other control methods is limited. This should provide a better opportunity for population densities to increase and disperse to

adjacent infestations and also enable the site to be used for the collection and transfer of the agent to other sites. This is particularly important for agents such as *S. staphylinus* that are initially slow to increase and disperse. However, if gorse is to be controlled in areas where the widely established *T. lintearius* and *E. ulicis* are present, no changes to control measures are required. This is because both agents have good dispersal abilities and can easily reinfest gorse if it is cleared from an area and starts to grow back.

As biological control is only one part of an integrated weed management strategy, studies are recommended that provide further information on the compatibility and impact of biological control and other control methods. As practices that reduce seedling survival are thought to be the most effective for gorse control (Rees and Hill, 2001), studies on gorse management would be most efficient if they focus on this part of the life cycle. This could include experiments to assess the impact that management practices (such as biological control using *S. staphylinus*, herbicide use, competition from pasture species and grazing management) have on gorse populations in a field environment.

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**Appendix 1. Development data used in Figure 3.1 and Table 3.3 for  
(a) *Phytoseiulus persimilis* (b) *Tetranychus urticae* and (c) *Tetranychus  
lutearius***

Note: Some studies in (a) included more than one strain fed on more than one diet. In these cases, means of the data were used and the number in parentheses following the development time is the number of strain/diet combinations comprising the mean.

**(a) *Phytoseiulus persimilis***

Temperature (°C)	Development time (days from egg to adult)	1/days	Reference
14	17.8 (4)	0.056	Davies (this study, Chapter 3)
15	18.7	0.053	Hamamura <i>et al.</i> (1976)
15	19.6	0.051	Sabelis (1981)
15	12.3 (3)	0.081	Galazzi and Nicoli (1996)
17.5	12.8	0.078	Hamamura <i>et al.</i> (1976)
20	7.2	0.138	Hamamura <i>et al.</i> (1976)
20	7.2	0.139	Sabelis (1981)
20	6.2 (3)	0.161	Galazzi and Nicoli (1996)
22.5	6.0	0.168	Hamamura <i>et al.</i> (1976)
24	5.8 (4)	0.172	Davies (this study, Chapter 3)
25	4.2 (4)	0.237	Escudero and Ferragut (2005)
25	4.9	0.206	Hamamura <i>et al.</i> (1976)
25	5.1	0.196	Badii and McMurtry (1984)
25	4.0	0.249	Toyoshima and Amano (1999)
25	4.5 (3)	0.221	Galazzi and Nicoli (1996)
26.7	4.1 (4)	0.242	Perring and Lackey (1989)
27.5	4.3	0.232	Hamamura <i>et al.</i> (1976)
30	3.5	0.285	Hamamura <i>et al.</i> (1976)
30	3.9	0.256	Sabelis (1981)
30	3.4 (3)	0.297	Galazzi and Nicoli (1996)

**(b) *Tetranychus urticae***

Temperature (°C)	Development time (days from egg to adult)	1/days	Reference
15	25.1	0.040	Bounfour and Tanigoshi (2001)
15	27.5	0.036	Herbert (1981)
15.5	25.3	0.040	Carey & Bradley (1982)
18	18.6	0.054	Herbert (1981)
18.3	16.5	0.061	Carey & Bradley (1982)
20	16.0	0.063	Bounfour and Tanigoshi (2001)
21	12.5	0.080	Herbert (1981)
21.1	15.0	0.067	Carey & Bradley (1982)
23.8	10.3	0.097	Carey & Bradley (1982)
25	12.3	0.081	Bounfour and Tanigoshi (2001)

**(c) *Tetranychus lintearius***

Temperature (°C)	Development time (days from egg to adult)	1/days	Reference
15	38.2	0.026	Stone (1986)
20	26.0	0.038	Stone (1986)
23	17.1	0.058	Stone (1986)
25	15.3	0.065	Stone (1986)

## Appendix 2. Publications and presentations during candidature

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Ireson, J.E., Davies, J.T., Kwong, R.M., Holloway, R.J. and Chatterton, W.S. (2006). Biological control of gorse (*Ulex europaeus*) in Australia: where to next? Proceedings of the 1<sup>st</sup> Tasmanian Weeds Conference, Launceston, Tasmania, pp. 15-19.

Davies, J.T., Ireson, J.E. & Allen, G.R. (2005). The impact of gorse thrips, ryegrass competition and simulated grazing on gorse seedlings performance in a controlled environment. *Biological Control*, 32: 280-286.

Davies, J.T. (2005). The impact of biological control on gorse. PhD seminar, New Town Research Laboratories – Oral presentation.

Davies, J.T., Ireson, J.E. and Allen, G.R. (2004). The role of natural enemies in regulating populations of biocontrol agents of gorse (*Ulex europaeus*). In: Sindel, B.M. and Johnson, S.B. (Eds.), 14th Australian Weeds Conference Proceedings. Charles Sturt University, Wagga Wagga, NSW, pp. 101-104 (Poster and paper).

Ireson, J.E., Kwong, R.M., Gourlay, A.H., Davies, J.T., Chatterton, W.S. and Holloway, R.J., (2004). Progress on the biological control of gorse (*Ulex europaeus*) in Australia. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K. (eds), Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO Entomology, Canberra, Australia, pp. 415-418.

Davies, J.T., Ireson, J.E. and Allen, G.R. (2004). The impact of gorse thrips, ryegrass competition and simulated grazing on the establishment and growth of gorse seedlings. XI International Symposium on Biological Control of Weeds, CSIRO Entomology, Canberra, Australia – Poster presentation.

Davies, J.T. (2003). The impact of gorse thrips, ryegrass competition and simulated grazing on the establishment and growth of gorse seedlings. Australian Entomological Society & 6th Invertebrate Biodiversity & Conservation combined conference. Hobart Tas – Oral presentation.