Multi-Species Interactions in Weed Biocontrol: *Carduus nutans* as a Case Study

---

A thesis submitted in partial fulfilment of the

requirements for the Degree

of Doctor of Philosophy in Ecology

in the University of Canterbury

by R. Groenteman

University of Canterbury

2007

Carduus nutans inflorescence. Photo by Matt Walters

Table of Contents

Table of Contents ..... **i**
Acknowledgements ..... **v**
Abstract ..... **vii**
Definitions ..... **ix**
Chapter 1: Introduction **1**
1.1 Insect herbivory, weed biocontrol and the challenge in demonstrating population level effects 1
1.2 Risks to non-targets, risks from invasives and the challenge in targeting 'sleeper weeds' 3
1.3 Predicting agent effectiveness 5
1.3.1 Predicting effects of interactions between biocontrol agents 6
1.4 Thistles as a case study 7
1.4.1 Why thistles 7
1.4.2 System organisms 8
1.5 Thesis rationale 10
Chapter 2: Which species of the thistle biocontrol agent *Trichosirocalus* are present in New Zealand? **13**
2.1 Introduction 13
2.2 Methods and Materials 14
2.3 Results and Discussion 15
2.3.1 Conclusions and Outlook 19
2.4 Addendum 19
Chapter 3: Abundance, phenology and impact of nodding thistle biocontrol agents on nodding thistle in New Zealand **21**
3.1 Introduction 21
3.2 Methods 23
3.2.1 *T. horridus* abundance in *C. nutans* rosettes 23
3.2.2 Seed predators effect on *C. nutans* 24
3.3 Results 27
3.3.1 *T. horridus* abundance in *C. nutans* rosettes 27
3.3.2 Seed predators effect on *C. nutans* 29
3.4 Discussion 40
Chapter 4: Interactions between nodding thistle biocontrol agents **45**

4.1 Interactions between nodding thistle seed predators 45
4.1.1 Introduction 45
4.1.2 Materials and methods 46
4.1.2.1 Plants and insects 46
4.1.2.2 Interactions between the two seed predators 47
4.1.2.3 Simulation of the effect of the two seed predators on a nodding thistle population 48
4.1.3 Results 48
4.1.3.1 Interactions between seed predators 48
4.1.3.2 Simulation of the effect of seed predators interaction on a nodding thistle population 49
4.1.4 Discussion 49
4.2 Interactions between nodding thistle biocontrol agents 51
4.2.1 Introduction 51
4.2.2 Methods 53
4.2.2.1 Plants and insects 53
4.2.2.2 Interactions between the three biocontrol agents: field experiment 54
4.2.2.3 Interactions between the biocontrol agents: simulation of biocontrol agents effect on *C. nutans* population growth rate 55
4.2.2.3 Statistical analysis 56
4.2.3 Results 57
4.2.3.1 Effects of *T. horridus* on *C. nutans* growth, survival and damage 57
4.2.3.2 Effects of *T. horridus* on *U. solstitialis* 61
4.2.3.3 Effect of *T. horridus* and *U. solstitialis* on *C. nutans* seed production 61
4.2.3.4 Simulation of the effect of the biocontrol agents on *C. nutans* population growth rate 62
4.2.4 Discussion 63
4.2.4.1 Effects of root herbivory by *T. horridus* on *C. nutans* 63
4.2.4.2 Effect of *U. solstitialis* on *C. nutans* seed production 65
4.2.4.3 Interactions between the root herbivore *T. horridus*. and the seed predator *U. solstitialis* 66
4.2.4.4 Conclusions 68
Chapter 5: Multi targeting for management of 'sleeper' weeds: the role of associational susceptibility to a shared herbivore 71
5.1 Introduction 71

5.2 Methods 74
5.2.1 Study system 74
5.2.2 Plants and insects 76
5.2.3 Biocontrol agent attack on non-target species in the presence and absence of *C. nutans*: a field experiment 76
5.2.4 Biocontrol agent attack on non-target species in the presence and absence of *C. nutans*: a field survey 78
5.2.4 Statistical analysis 79
5.2.5 Study limitations 82
5.3 Results 82
5.3.1 Field experiment 82
5.3.2 Field survey 93
5.4 Discussion 96
5.4.1 Conclusions 99
Chapter 6: Factors affecting oviposition rate in the weevil *Rhinocyllus conicus* on non-target *Carduus* spp. in New Zealand 103
6.1 Introduction 103
6.2 Materials and Methods 103
6.3 Results and Discussion 104
Chapter 7: Changes in thistle abundance in Canterbury, New Zealand, 1985-2007 in relation to biocontrol 109
7.1 Introduction 109
7.2 Method 110
7.3 Results 113
7.4 Discussion 123
7.4.1 Thistle life strategies effect on year-to-year variation 123
7.4.2 Densities and size in relation to life strategy 124
7.4.3 Densities and size in relation to farm management 125
7.4.4 Success of biocontrol on *C. nutans* 126
Chapter 8: Synthesis 129
8.1 Population models - a tool for improving effectiveness in weed biocontrol 129
8.2 Sleeper weeds and multi targeting 132
8.3 Patterns of herbivory on multiple plant species and multi targeting 133
References 137
Appendix A: Supplementary statistical analysis for Section 4.1 155

Appendix B: Supplementary material for chapter 5 157

Appendix C: Multiple species density estimation using the truncated joint point-distance nearest-neighbour distance method 163

Acknowledgements

To my supervisors, Prof. Dave Kelly, Dr. Simon Fowler and Dr. Graeme Bourdôt – thank you for an inspiring and stimulating experience. Each of you in your own special way made me feel you wanted to see me blossom. Dave, I am grateful for your mentorship, your contagious enthusiasm, your confidence in me and your healthy attitude to science and to life in general (I admit I wasn't that good a student on that…). Graeme and Simon, thank you for your wonderful ideas, your quick responses to my needs – be it in the field season or by commenting on drafts, and for making me feel my work is meaningful and appreciated. I thank you all for providing such a supportive environment, and for preparing me well for my next journey!
I thank Dr. Jo Ward who was on the supervising committee for her support throughout.

Various people contributed their expertise to the nation-wide *Trichosirocalus* survey in Chapter 2: Lynley Hayes coordinated the survey; Stephen Thorpe guided me on the weevil identification; and Clair Galilee provided technical assistance. Bridget Keenan, Greg Hoskins, Harvey Phillips, Lindsay Grueber, Neil Gallagher and Malinda Matthewson, collected weevil samples, and Murray Turner did an archival search for clues in this detective story; John Marris kindly allowed me to dissect the weevils collected in 1990; Andy Sheppard, David Briese, Anthony Swirepick, Tim Woodburn, and Miguel Alonso-Zarazaga helped revealing the Australian side of the story, and Rolf Oberprieler kindly compared New Zealand specimens to the Australian paratypes; I thank Andy and Rolf for insightful discussions on the taxonomic complications involved with the species complex.

Kat Bushman, Morgan Coleman, Mike Cripps, Sarah Luxton and Caroline Thomson joined me on wintry days to collect thistle rosettes for the *T. horridus* survey in Chapter 3, and Caroline also helped dissecting them. Kathrin Affeld, Kat Bushman, Morgan Coleman, Emri Dolev, Steve Hough, Helen Parish, Kate Rose, Caroline Thomson, Eyal Twig, Amit & Gal Yigal-Bruner, and the Groenteman and Carasuk parents helped in the field experiments in Chapters 4, 5 & 6. Janette Beaton, Merodie Beavon, Chris Berg, Morgan Coleman, Jenny Gerson, John Hanckock, Jennie Iles, Nicolette LeCren, Ben Myles, Helen Parish, and Caroline Thomson dissected thousands of thistle capitula from the field experiments in Chapters 4, 5 & 6. Laura Bellaney, Raviv Carasuk, Morgan Coleman, Nicolette LeCren, Fraser Maddigan, Ceisha Poirot, Marcus Provis, Lindsay Smith, Tom Sullivan, Chris Summers and Julia Wilson-Davey all took part in the field surveys presented in Chapter 7 between 2004-05 and 2006-07.
Nicolette LeCren and Hagar Shaviv did solid weeks of data entry!
The co-authors on the papers which comprise Chapters 2 and 6 and Section 4.1 are my supervisors, who have contributed ideas for the research and have revised the manuscripts.

Lindsay Smith tolerated me and my crazy needs (giving each thistle a shower where there's no water supply, to mention one…), always positive and packed with good humour – I honestly wouldn't have got this far without your help!

Maggie Tisch and Jenny Ladley, for your caring and your endless smiles, for always having a solution, a great idea, a helping hand and moral support; and for your recruitment to sew those cages… A huge thank you.

To all my great lab and office mates: Debra Wotton, Melissa Hutchison, Laura Young, Michal Sarfati, Jenny Ridgen, Chucky Van-Schravendijk, Margee Will, Sara-Jane O'conor, Merodie Beavon and Marc Thomas, thank you for the moral support along the way and for helping through paths in a new country. I am so lucky to have met you!

Thanks to Kat Shea's lab members – to Kat, for useful discussions, for your brilliant ideas, for guiding me into matrix modelling (even though I still have a long way to go), and for being so supportive; Zeynep Sezen, for happily sharing your experience in growing thistles and weevils and your R code; and Eelke Jongejans, for kindly providing the R code for the matrix population model.

Dave Conder helped to maintain (and eventually to get rid of) many thousands of thistles at the University of Canterbury glasshouses. Stuart Oliver and the grounds team at Landcare Research and Geoff Hurrell provided much support and friendly advice at the experimental sites.

Jan McKnzie, Neil Andrews and Matt Walters assisted with imaging. Lindsey Milbrath shared tips on harvesting *T. horridus* eggs. Mark McNeill showed me potential non-target parasitoids of the weevils in this study. Les Batcheler clarified the modification of the nearest neighbour method to estimate densities of multiple species populations.

To all the farmers who let me sample on their properties, and especially Baven Grice and Cheryl Ridgen. Also, thanks to Phil Crotty and Kevin Loe who directed me to some sites.

Dave Saville, Richard Duncan Ottar Bjørnstad and Jason Tylianakis gave invaluable statistical advice and Richard and Ottar assisted tremendously with frustrating R coding.

An exemption to grow *C. nutans* plants was granted by Environment Canterbury (reference number BI2T/1).

The project was funded by the New Zealand Foundation for Research Science and Technology (Outsmarting Weeds programme, contract number C10X0318); Brian Mason Scientific & Technical Trust funded the thistle population surveys in 2005-06 and 2006-07.

Lastly, I wish to thank my family – my parents, Pnina and Alex Groenteman and Shlomit and Meir Carasuk, for your enormous emotional and physical support, and for the genuine interest in what exactly is it that I find exciting about bugs and prickles; at least you got to see some of New Zealand out of this whole adventure… And Raviv – for tolerating me, for feeding me, for caring, and for being there, by my side, in this journey, and in others. Thank you.

Abstract

Classical biocontrol systems are sometimes treated as an exercise in community assembly. As such, they include multiple species interactions. This thesis explores multi-species aspects in classical weed biocontrol, using thistles as a case study.

The abundance, phenology and impact of three biocontrol agents were followed on their target host, *Carduus nutans* L. and are described, for the first time in New Zealand for two of them (*Urophora solstitialis* L. and *Trichosirocalus horridus sensu* (Panzer)).

Composition in New Zealand of the recently revised *Trichosirocalus* weevil species complex was surveyed nation-wide. One species only was found, albeit exhibiting a wider host range than anticipated from the published revision.

Interspecific interactions and individual and combined effect of multiple biocontrol agents on *C. nutans* were tested in cage setups; the effect on the weed population was then estimated by manipulations of an existing matrix population model for this weed in New Zealand. The potentially better seed predator (*U. solstitialis*) was outcompeted by the worse seed predator (*Rhinocyllus conicus* (Froehlich)) which has similar niche preference. *Urophora solstitialis* was also adversely impacted by the crown-root feeder (*T. horridus*). *Trichosirocalus horridus* affected *C. nutans* survival, even at the medium density used, and significantly reduced potential seed production by 33%; in field densities, *T. horridus* is likely to affect *C. nutans* even more. *Urophora solstitialis* was estimated to destroy about 28% of the remaining seed in the absence of the other agents, and about 17% in the presence of *T. horridus*. The estimated combined effect of *T. horridus* and *U. solstitalis* on *C. nutans* population growth rate was greater than the effect of either agent alone.

In the face of growing weed invasions, multiple thistle species were used to test 'multi-targeting' as a novel approach to target groups of 'sleeper weeds'. Both in a field experiment and in a field survey, the seed predator *R. conicus* was found to attack and damage some 'non-target' thistle species more in the presence of the target species (*C. nutans*) than in its absence; however, levels of attack on non-target species were always modest.

The ultimate goal of biocontrol is to reduce weed populations. A field survey revealed that current population densities of multiple thistle species in Canterbury are not obviously lower than in the mid 1980s, when only *R. conicus* was present. This may be because successful biocontrol has reduced the management input required to maintain the same thistle density.

Definitions

The area of plant invasions suffers from mixed use of terms. For the purpose of this work I use terms as suggested by Richardson *et al.* (2000, p. 98):

Alien (also exotic, non-native) **plants** – Plant taxa in a given area whose presence there is due to intentional or accidental introduction as a result of human activity.

Naturalised plants – *Alien plants* that reproduce consistently and sustain populations over many life cycles without direct intervention by humans (or in spite of human intervention); they often recruit offspring freely, usually close to adult plants, and do not necessarily invade natural, seminatural, or human-made ecosystems.

Invasive plants – *Naturalised plants* that produce reproductive offspring, often in very large numbers, at considerable distance from parent plants, and thus have the potential to spread over a considerable area. In some cases, plant species that have self-introduced without human agency may be considered invasive, especially if they have a negative effect on endemic species (R.G.).

Weeds – plants (not necessarily *alien*) that grow in sites where they are not wanted and which usually have detectable economic or environmental effect.

Thistle capitula throughout this work were harvested when ripe. A capitulum was considered **ripe** when it has finished flowering, the florets have turned brown and the capitulum itself has started turning brown, but no seed or pappus has abscised.
Capitula were collected at this stage to allow full seed development; the receptacle feeding biocontrol agents attack takes place much earlier – at the bud stage.

Damage ranking. Throughout this work I have ranked damage caused to thistle receptacle tissue as zero, little, medium and high. These correspond with:
Little – up to 10% of the receptacle damaged
Medium – between 10 and 50% of the receptacle damaged
High – more than 50% of the receptacle damaged

Throughout this work I use the terms ‘primary’, ‘secondary’ and ‘tertiary’ to describe the position of thistle capitula (or clusters of capitula in the case *Carduus pycnocephalus* and *Carduus tenuiflorus*). **Primary** position is at the terminus of the main stem and of the stems branching off the main stem; **secondary** position is at the terminus of subsidiary branches; and **tertiary** position is at the terminus of branches branching off the subsidiary ones. These positions are illustrated in the following diagram:

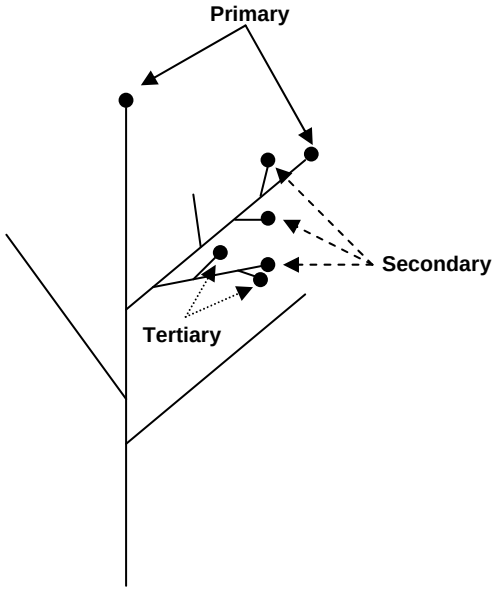

Illustration of a thistle plant indicating capitula position-types.

Chapter 1: Introduction

Classical biological control has been described as representing "an applied analogue to a basic theme in ecology, *assembly dynamics*" (Holt & Hochberg, 2001, p. 15). Indeed, rarely can biocontrol systems be simplified to the microcosm of a single pest and a single agent; rather, a community is assembled, and multiple species are involved.

This thesis explores different aspects of multiple species involvement in a weed biocontrol system. In so doing, it delivers on some of the core challenges weed biocontrol faces as a field of applied science. I used thistles as a study system to address challenges such as demonstrating population level effects of biological control on target weeds, improving our ability to predict agents' effectiveness, and seize the risk we face from 'sleeper weeds'. The study of biocontrol agents impact on their target thistles in this thesis progresses from the individual capitulum (seed head) level, through the individual plant, to population level simulations and actual field population level assessment.

1.1 Insect herbivory, weed biocontrol and the challenge in demonstrating population level effects

For a long time insect herbivores were considered unimportant in plant population dynamics (Hairston *et al.*, 1960), be it due to top-down regulation by natural enemies or to bottom-up regulation through resource available to their plant hosts (summarized in Crawley, 1997). However, more recent research has shown that herbivores can affect individual plant performance, change plant population dynamics, and shape plant communities (some examples include Louda, 1982a, b; Brown *et al.*, 1987; Louda *et al.*, 1990; Sheppard *et al.*, 1990; Rees & Brown, 1992; Louda & Potvin, 1995; Louda & Rodman, 1996).

Insect herbivores have been intentionally used in classical biological control of weeds since the early 1900s (Goeden, 1988; Julien & Griffiths, 1998), with some phenomenal success stories (Goeden, 1988; McFadyen, 1998; McFadyen, 2003), but also with numerous successful establishments of agents which show no effect on their target weed (Crawley, 1989; Julien & Griffiths, 1998; Denoth *et al.*, 2002; McFadyen, 2003; McClay & Balciunas, 2005). While past weed biocontrol research focused on (apart from safety and host specificity) agent successful establishment and impact on the individual plant, it is now well realized that even substantial damage to the individual plant does not necessarily translate to a population level effect (Crawley, 1988, 1989; Turnbull *et al.*, 2000; McClay & Balciunas, 2005). Seed predators have raised special controversy: weeds are often characterised by high reproductive capacity and reducing this capacity is often the target of biocontrol programmes;

if the weed populations are seed limited, even relatively minor seed predation could result in their decline (Janzen, 1971; Louda & Potvin, 1995); yet, in non-seed-limited plant populations, an agent that consumes even the vast majority of the entire seed crop might have no population-level impact (Janzen, 1971; Sheppard *et al.*, 1994). A matrix population model predicted that 69% seed reduction would bring *Carduus nutans* L. (nodding/musk thistle) populations in New Zealand to decline (Shea & Kelly, 1998); much higher seed reductions have been reported by *C. nutans* seed predators elsewhere (e.g., Woodburn & Briese, 1996), but not in New Zealand. Matrix population models offer a robust method of linking of the individual (nodding thistle plant in this case) and the population around a simple description of the life cycle. Life cycle can be described in terms of age, size or their combination. The dynamics of the population is determined through the vital rates: birth, growth, maturation, fertility and mortality. The matrix is comprised from the probabilities of individuals in the population to transit from each stage (size or age group) to the next over one time unit. The matrix allows calculation of the population growth rate. In addition, it allows identifying the transitions which contribute most to this growth rate and the transitions which are most vulnerable to perturbation, and which may be the most suitable to target in a biocontrol programme (Caswell, 2001; Shea & Kelly 1998).

To assess and judge the cause-effect relationship between biological control agents and changes to weed status, long-term ecological research is required, that monitors weed populations. This aspect has often been commented on as neglected in biocontrol programmes (Simberloff & Stiling, 1996a; Crawley, 1997; McFadyen, 1998; Fowler *et al.*, 2000; Delfosse, 2005), ultimately for funding reasons; funding agencies usually prefer addressing the next weed problem than keeping the spending going on weeds that are no longer a problem (McFadyen, 1998; Fowler *et al.*, 2000; Delfosse, 2005). Biocontrol practitioners value long term weed population monitoring for the purpose of hypothesis testing, for learning about interactions within the biocontrol system, for improving the practice, for demonstrating biocontrol benefits and for justifying future biocontrol programmes; yet funding for this type of study is rarely available (Fowler *et al.*, 2000; Delfosse, 2005).

This thesis formed a unique opportunity to assess the population density of thistles in Canterbury two decades after a similar assessment took place (Bourdôt & Kelly, 1986). Within this time two biocontrol agents have been introduced to New Zealand against nodding thistle (on top of one agent which was present at the time of the original assessment). Chapter 7 of this thesis presents this population level assessment, and attempts to relate the status of thistle populations to biocontrol agents' impact.

1.2 Risks to non-targets, risks from invasives and the challenge in targeting 'sleeper weeds'

The risk of adverse effects to non-target species introduced as classical biological control agents has received much attention both from biocontrol critics (Howarth, 1983, 1991; Simberloff & Stiling, 1996a; Strong, 1997; Simberloff & Stiling, 1998; Strong & Pemberton, 2000; Louda *et al.*, 2003a, b) and supporters (Huffaker, 1959; Zwölfer & Harris, 1971; Harris, 1991; Frank, 1998; McFadyen, 1998; Fowler *et al.*, 2000; Fowler *et al.*, 2001; Sheppard *et al.*, 2003; Delfosse, 2005; Fowler & Withers, 2006). Improving predictability of non-target effects in classical weed biocontrol is constantly called for (e.g., Louda *et al.*, 2003a, b; Louda *et al.*, 2005). Cases of non-target effects receive high-profile publicity (e.g., Louda *et al.*, 1997; Strong, 1997). Demands are raised for a risk-free practice in which, not only the physiological host range, but also the ecological (Louda *et al.*, 1997; Louda *et al.*, 2003a, b) and chemical (Jordon-Thaden & Louda, 2003) host range, as well as every possible indirect interaction in any possible community a mobile biocontrol agent may independently disperse to, are known before the agent is approved ("guilty until proven innocent", Simberloff & Stiling, 1996a, p. 1970; Simberloff & Stiling, 1996b; Louda *et al.*, 2003a; Louda & Stiling, 2004). Such demands, if they ever become requirements for the release of biocontrol agents, would practically terminate biocontrol releases (Fowler *et al.*, 2000; Sheppard *et al.*, 2003). Regardless, host specificity tests constantly improve, and both physiological and ecological aspects that would not have been included in early days, are now an integral part of current protocols (Wapshere, 1974; Harris, 1991; McFadyen, 1998; Thomas & Willis, 1998; Fowler *et al.*, 2000; Sheppard *et al.*, 2003; Fowler *et al.*, 2004; Paynter *et al.*, 2004; Delfosse, 2005). In addition, the process is more open for public comment (Sheppard *et al.*, 2003; Barratt & Moeed, 2005; Julien *et al.*, 2007). However, more rigorous host specificity tests and addressing public submissions also take longer and are costly (Fowler *et al.*, 2000; Sheppard *et al.*, 2003; McClay & Balciunas, 2005; Julien *et al.*, 2007).

The North American case of non-target effects by the thistle seed predator *Rhinocyllus conicus* (Froehlich), a biocontrol agent of *C. nutans*, warrants mentioning both for the perturbations it caused (and still causes) in the field of classical biocontrol, and for its relevance to the system studied in this thesis. The request for approval to release the weevil in the United States clearly stated that native North American *Cirsium* spp. thistles could support weevil development (e.g., Zwölfer & Harris, 1984). The risk to native thistles was viewed acceptable by all reviewers, given the expected benefits from the likely control of this major weed, and approval to release was given (Delfosse, 2005). More than two decades after release, *R. conicus* was documented extensively attacking threatened native North American

Cirsium spp. in a high-profile case (Louda *et al.*, 1997; Strong, 1997). *Rhinocyllus conicus* has since featured (mainly as a villain) in numerous publications, and the controversy surrounding this agent is only increased by the fact it does not always suppress its target weed very effectively (e.g., Kelly & Wood, 1991). The two main messages emerging from this case in regards to non-target effects are that (a) host specificity tests for this agent were remarkably complete (especially considering that they took place in the early 1960s) and that the host range expansion was anticipated (Gassmann & Louda, 2001; Arnett & Louda, 2002; Delfosse, 2005); and that (b) values held by society as warranting protection, and human perceptions as to what is an acceptable risk, change (Delfosse, 2005). Nowadays, attempts to identify potential future conflicts of interests are an integral part of weed biocontrol, at least in New Zealand (e.g., Stanley & Fowler, 2004). Without undermining their value, and in the spirit of conflicting views and perceptions, it is worth noting that the native North American thistles are considered weeds by some parts of society (e.g., Lamp & McCarty, 1982b; Guretzky & Louda, 1997).

In reality, no amount of time would be sufficient to enable us to predict all possible direct and indirect impacts of an alien organism in a new environment (Fowler *et al.*, 2000; Fowler *et al.*, 2001; Moran *et al.*, 2005), be it a pest or an intentionally introduced biocontrol agent. Classical biocontrol introductions are deliberate invasions, and are not risk-free (Fowler & Withers, 2006); however, while agent releases are delayed by extended host testing and public hearings, exotic plants continue to become naturalized and invasive worldwide (Culliney, 2005; Moran *et al.*, 2005; Sullivan *et al.*, 2005; Lambdon & Hulme, 2006; Bourdôt *et al.*, 2007; Julien *et al.*, 2007), inflicting changes to ecosystems (Fowler *et al.*, 2001; Crooks, 2002; Sheppard *et al.*, 2003; Stanley & Fowler, 2004; Culliney, 2005; Delfosse, 2005; Moran *et al.*, 2005), sometimes to the point where intervention is too late (Crooks, 2002). Hence there are both costs from too-hasty biocontrol agent release, and (less often recognised) costs from delays in agent release.

New Zealand is under particular risk from the increasing number of invasive species (Williams & Lee, 2001; Julien *et al.*, 2007). Which naturalized alien is going to become the next weed is hard to predict; yet some groups of plants are more likely candidates than others. Naturalized species in these groups can be regarded as possible ‘sleeper weeds’, which are likely to become invasive in due course. In this thesis I test whether non-target effects are necessarily the Achilles’ heel of biological control (Louda *et al.*, 2003b), or whether the vast theory accumulated from studies of non-target effects could be channelled to beneficial paths such as targeting multiple ‘sleeper weeds’.

1.3 Predicting agent effectiveness

Biological control of weeds has gone a long way in predicting the host range of candidate agents. It is agreed that we are actually very good at this (Harris, 1981; McFadyen, 1998; Pemberton, 2000; Arnett & Louda, 2002; Louda *et al.*, 2003b). We have been far less successful, however, in increasing our predictive power of agent effectiveness (Harris, 1981; Myers, 1985; Denoth *et al.*, 2002; Haefliger *et al.*, 2006; Raghu & Van Klinken, 2006; Gerber *et al.*, 2007a), despite decades of theoretical and empirical attempts (Harris, 1981; Myers, 1985; Blossey, 1995; McEvoy & Coombs, 1999; Balciunas, 2004; McClay & Balciunas, 2005; Briese, 2006; Van Klinken & Raghu, 2006; Gerber *et al.*, 2007a). Agent effectiveness has always been important to agent selection (Harris, 1973, 1981; Goeden & Kok, 1986; McClay & Balciunas, 2005). However, it tended to drop in hierarchy against host specificity requirements and practicality of agent transportation and rearing (Sheppard, 2003; McClay & Balciunas, 2005; Raghu *et al.*, 2006). Predictive ability of agent effectiveness could benefit biocontrol in many aspects. Firstly, the time to achievement of successful control could be critically shortened. This is well illustrated in the well-known example of the moth *Cactoblastis cactorum* (Bergroth), introduced to Australia for biological control of prickly pear cactus *Oputina stricta* (Haworth). The moth was introduced 12 years into the programme, 11th of 21 agents, and was the most effective of all agents (summarized in Raghu & Van Klinken, 2006). Had *C. cactorum* effectiveness been predictable, *O. stricta* control could possibly have been achieved much sooner. Secondly, the cost of biocontrol programmes could be drastically reduced (and benefit-cost ratio increased) if effectiveness could be predicted, and only effective agents tested for host specificity (McClay & Balciunas, 2005). Thirdly, the risk for non-target effects would decrease. Ineffective agents which do not suppress their target weed can sustain high populations, and could indirectly affect food webs (McEvoy & Coombs, 2000; Holt & Hochberg, 2001).

Predicting agent effectiveness was recently identified as one of the biggest challenges for weed biocontrol scientists applying ecological theory (Raghu & Van Klinken, 2006). While prediction prior to introduction is most desirable, we have still much knowledge to gain from past introductions that could be applied in the future, in an active adaptive management strategy (Shea *et al.*, 2002; Shea & Kelly, 2004).

Interactions between multiple biocontrol agents are a significant aspect of agent effectiveness, which bears much room for improvement.

1.3.1 Predicting effects of interactions between biocontrol agents

A common paradigm in biological control of weeds is that more than one agent is required to produce successful control. Two differing hypotheses lead to this paradigm: (a) that multiple sources of stress (which could be generated, for example, by multiple biocontrol agents) are usually necessary to sufficiently affect the weed (Harris, 1981, 1991; Tipping, 1992), and (b) that only one agent is required for successful control but, not knowing in advance which is the 'silver bullet' agent, multiple introductions increase the chances of it actually being introduced (the 'lottery ticket' model, Myers, 1985). Evidence exists to support both hypotheses, and the fact remains that introduction of multiple agents improves programme success rate (Denoth *et al.*, 2002).

It was believed in the past that a better biocontrol agent is, by definition, also a better competitor; subsequently, if competition between agents resulted in the displacement of either, the better biocontrol agent was alleged to outcompete the less effective agent (Huffaker & Kennett, 1969). If this was the case, there would be no risk to the overall level of biocontrol from introducing agents that later turn out to be less effective.

Competitive interactions between phytophagous insects are notably common amongst phytophages feeding in discrete niches, such as seed predators, and amongst phytophages in introduced systems, such as biological control systems, where the effects of natural enemies are often excluded (Denno *et al.*, 1995). Examples do exist of improved control by potentially competitive agents. Even closely competing agents may have a complementary effect, with their combination resulting in a stronger effect on the weed than with either species alone (e.g., Berube, 1980; Myers & Harris, 1980; Hoffmann & Moran, 1992; Maron, 1998). Nevertheless, it is nowadays realized that the better competitor and the better control agent may not be a single entity, and that competitive interactions between biocontrol agents could jeopardise control efforts. Briggs (1993), in modelling interactions between parasitoids, showed that a better competitor could exclude a better biocontrol agent by, for example, attacking earlier in the life cycle of the host, such as the non-damaging egg stage, thus excluding parasitoids of later stages, while not necessarily having an effect on the density of later, damaging, life stages. In weed biocontrol too, there exist empirical examples of competitive interactions which negatively affect control efforts between agents that attack similar plant parts (e.g., Woodburn, 1996a; Crowe & Bourchier, 2006), and between agents that attack different plant parts, at different times (e.g., Milbrath & Nechols, 2004a). It was wisely suggested that amongst multiple potential biocontrol agents, the species expected to be

a better competitor should only be introduced if the lesser competitor has not been able to achieve control goals (Zwölfer, 1973; Harris, 1989a).

Testing for competitive interactions between potential agents in the country of origin, prior to introduction, is highly valuable in suggesting the sequence of agent introduction that is likely to produce the best control outcome (Davis *et al.*, 2006). However, such tests are still relatively uncommon (Gerber *et al.*, 2007a), and cannot guarantee that results would be replicated in the new environment of the invaded range; nonetheless, we can greatly benefit from better understanding of competitive interactions between agents post introduction.

Carduus nutans biocontrol agents in New Zealand present a particularly challenging system, with potential direct competitive interactions between the two seed predators (*R. conicus* and *Urophora solstitalis* L.), as well as potential indirect interaction between the seed predators and the spatially and temporally separated root feeder (*Trichosirocalus horridus sensu* (Panzer)) (for clarification see Chapter 2). None of these interactions have been studied in New Zealand, (except in a short survey of *R. conicus* and *U. solstitialis* as part of a study by McNeill & Fletcher, 2005), and only some (the interaction between *T. horridus* and *R. conicus* and between *R. conicus* and *U. solstitialis*) have been studied elsewhere (Tipping, 1992; Woodburn, 1996a; Milbrath & Nechols, 2004a).

1.4 Thistles as a case study

1.4.1 Why thistles

Thistles of the plant tribe Cardueae are represented in the New Zealand flora by at least 115 species, all of which are alien. At least nine species are economic weeds, and dozens other species have been recorded as weeds elsewhere, some of which are already naturalized here and thus may be 'sleeper weeds'.

Three of the economically harmful thistle species are currently under biological control programmes in New Zealand (all three are still significant economical weeds), the longest of which, against *C. nutans*, dates back to the early 1970s, with partial success to date. Furthermore, a robust matrix population model which was developed for *C. nutans* in New Zealand (Shea & Kelly, 1998) and was recently updated (Jongejans *et al.*, 2008) makes this a highly informative study system. Therefore, thistles in New Zealand represent a challenging group of weeds, with much scope for improvement of biocontrol, and unfortunately, with much scope for weediness (and future biocontrol needs).

1.4.2 System organisms

1.4.2.1 Thistles (Asteraceae)

Carduus nutans L. is a monocarpic annual or facultative-biennial herbaceous weed of Eurasian origin. Seedlings form flat rosettes which, after fulfilment of age and vernalization requirements, bolt (elongate a stem), flower and subsequently die (Medd, 1977). The taxonomy of the species is complex, and hybridization with *Carduus acanthoides* L. is possible, but most specimens in New Zealand are treated as *C. nutans sensu stricto* (Webb *et al.*, 1988; Popay & Medd, 1990).

Carduus nutans was first recorded in New Zealand in 1899 (Kirk, 1899). By 1940 it had already become a problem and was got mentioned in an act of Parliament (Allan, 1940). Like other thistles, it is assumed to have entered New Zealand as contaminants in imported seed (Jessep, 1990). Flowering usually begins in late November (spring), peaks around January, and depending on weather is largely completed by March but can continue well into June (winter) (Webb *et al.*, 1988; Popay & Medd, 1990; current study), or even longer (pers. obs.).

Carduus pycnocephalus L. and *Carduus tenuiflorus* Curtis are annual species, which share a similar demography; they germinate in autumn and produce numerous clusters of small seed heads (capitula) from November to January, with a flowering peak around December (Kelly, 1988; Webb *et al.*, 1988; pers. obs.). *Carduus pycnocephalus* and *C. tenuiflorus* were recorded as “common” and “very plentiful” in Otago by Thomson in 1894 (Allan, 1940, p. 169). They have already become a problem and got mentioned in an act of Parliament by 1940 (Allan, 1940)

Cirsium vulgare (Savi) Tenore is a facultative-biennial, which grows to sizes similar to those of *C. nutans*, and produces large capitula, similar in size to those produced by *C. nutans*. Flowering occurs from November to March, and peaks around January (Webb *et al.*, 1988; pers. obs.). Under the older synonym *C. lanceolatum* (L.) Hill, it was recorded as “common to locally abundant” by Hooker in 1867 (Allan, 1940, p. 169). By 1940 it had already become a problem and got mentioned in an act of Parliament (Allan, 1940). *Cirsium vulgare* has recently become the target of a biocontrol programme in New Zealand (Fowler *et al.*, 2000).

1.4.2.2 Biocontrol agents

Rhinocyllus conicus (Froehlich) (Coleoptera: Curculionidae), nodding thistle receptacle weevil, was introduced to New Zealand in 1972 (Jessep, 1989a). Adults emerge from overwintering in early spring to feed on thistle foliage and stem, feeding which is not particularly damaging to the plant, but is essential for egg maturation (Zwölfer & Harris, 1984). Adults are active for a period of about eight weeks (Zwölfer & Harris, 1984; Milbrath & Nechols, 2004a; pers. obs.). Jessep (1975) recorded an 18 week oviposition period in New Zealand, but I suspect this includes a partial second generation in the warmer North Island (Zwölfer & Harris, 1984, p. 43). A second generation can occur if larvae complete development when daylight is longer than 16 h, but for the most part, there is just one generation per year (Zwölfer & Harris, 1984). Eggs are deposited on the external lower surface of the involucre bracts of thistle flower buds, each egg covered by a cap made of masticated, cemented host plant particles (Zwölfer & Harris, 1984). In the native range, normally no more than 30 eggs per capitulum are deposited (Zwölfer & Harris, 1984); but in the introduced range, early capitula may receive hundreds of eggs each (Zwölfer & Harris, 1984; Jessep, 1989a; Woodburn, 1996a). Larvae hatch after 6-9 days and tunnel their way into the receptacle where they feed, for four larval instars, on callus induced by their tunnelling; this feeding mode prevents seed development (Zwölfer & Harris, 1984; Harris & Shorthouse, 1996). Pupation occurs within the thistle capitulum, and total development from egg to adult lasts 45 to 55 days (Zwölfer & Harris, 1984). At one time, the species was suggested to consist of several biotypes (Goeden & Ricker, 1978) or ecotypes (Zwölfer & Preiss, 1983) with distinct host preferences and failure to develop on part of the acceptable host range for *R. conicus* as a whole; an allozyme work later distinguished two subspecies – a temperate one and a Mediterranean one, and suggested that the species *Rhinocyllus oblongus* Capiomont & Leprieur was, in fact, the Mediterranean *R. conicus* subspecies (Klein & Seitz, 1994). The population introduced to New Zealand originates from the (temperate) Upper Rhine Valley in Germany (Jessep, 1989a).

Trichosirocalus horridus sensu (Panzer) (Coleoptera: Curculionidae), nodding thistle crown weevil, was introduced to New Zealand in 1982, from German origin (Jessep, 1989a). The species was recently revised and is now considered a complex of at least three species (Alonso-Zarazaga & Sánchez-Ruiz, 2002). The species composition in New Zealand is examined in this thesis. Adults emerge in spring to feed on bolting thistle plants for 2-3 weeks before going into aestivation (summer dormancy). Adults reappear in autumn, when they feed and reproduce; batches of 1-6 eggs are deposited mainly into the midribs of thistle leaves (but

also in the leaf lamina) during autumn and winter (rosette stage). Larvae reach the centre of the rosette and the crown, where their feeding on meristematic tissue destroys growing tips. After three larval stages the larvae leave the rosette to pupate in the soil. There is one generation per year (Boldt & Campobasso, 1981; Woodburn, 1997).

Urophora solstitialis L. (Diptera: Tephritidae), nodding thistle gall fly, was introduced to New Zealand in 1992 from a French origin (Hayes, 2007). Adults start emerging from galls in early spring, after overwintering at the third larval instar, and continue emerging for about six weeks (Woodburn, 1996b). Females are ready for mating and oviposition upon emergence, and eggs are laid singly into developing floral tubes in closed thistle buds (Woodburn, 1996a, b). Larvae spend their first instar inside the developing ovule and then eat their way down to the receptacle, where they induce the formation of a lignified woody gall (Freidberg, 1984; Moeller-Joop & Schroeder, 1986). Larvae spend about four weeks feeding and the pupal stage lasts about two weeks (Persson, 1963). The gall induced by *Urophora* spp. flies is a powerful metabolic sink that drains resources form the plant as a whole (Harris, 1989b). *Urophora solstitialis* has two generations a year.

1.5 Thesis rationale

The overall aim of this thesis was to address some of the core challenges facing weed biocontrol systems in multi-species environments, using thistles as a case study. The thesis progresses from the fundamental issues of agent identification, abundance and impact, to analysis of interspecific interactions and their potential population level effects on the host. It then asks how agents with a preference for one weed host may affect multiple other closely related hosts, and how this can be used to improve biocontrol, especially of 'sleeper weeds'. Finally, it compares populations of multiple thistle species in the early days of *C. nutans* biocontrol programme and at present.

Just how critical correct identification of organisms in biocontrol systems is, is well demonstrated in past and present cases (Thomas & Room, 1986; Goeden, 1988; Ding *et al.*, 2006; Morin & Edwards, 2006). Given that taxonomy occasionally gets revised, re-identification well into a biocontrol programme is no less important than identification prior to introduction of an agent. Following the revision of *T. horridus* (Alonso-Zarazaga & Sánchez-Ruiz, 2002), it was believed that the fauna in New Zealand is likely to consist of either one or two of the newly and/or redescribed species. A nation-wide survey of *Trichosirocalus* sp. fauna, which is described in Chapter 2, aimed to resolve paradoxes arising

from the species revision. This chapter has been accepted for publication (Groenteman *et al*., in press b) and is presented in its accepted version, accompanied by an addendum to encompass developments which took place after manuscript acceptance.

Regardless of the identification outcome, three *C. nutans* biocontrol agents are already in the country. The last was introduced almost 18 years ago; yet, with the exception of several studies following the introduction of *R. conicus* (Kelly & McCallum, 1990; Kelly *et al.*, 1990; Kelly & McCallum, 1995; Shea & Kelly, 1998), there has been no principal study on the abundance, phenology or impact of these biocontrol agents. Chapter 3 follows the three biocontrol agents (abundance of *T. horridus* and abundance, phenology and impact of *R. conicus* and *U. solstitialis*) on *C. nutans* at the plant level, in field surveys and as a by-product of a field experiment.

Testing for possible interactions between potential biocontrol agents prior to introduction is excellent practice. However this is still the exception rather than the rule (Gerber *et al.*, 2007b). Still, much can be gained from studying interactions in the system after agent introductions, and refining of control strategies can continue in an active adaptive framework. Chapter 4 investigates interspecific interaction between the three *C. nutans* biocontrol agents already in New Zealand, for the first time, 18 years after the last of their introductions, and asks whether their combination is more (or less!) effective than their individual effects. This question is treated experimentally at the plant level, and inferences to the population level are made through manipulations of *C. nutans* matrix population model. One section of this chapter (Section 4.1) has been published (Groenteman *et al*., 2007). This section is presented as a modified version of the published paper which had to follow strict space limitations. It is complemented by an appendix (Appendix A) which details the statistical analysis to back results presented in the manuscript.

The effectiveness of biocontrol needs to be radically improved in the face of future invaders - 'sleeper weeds'. In Chapter 5, the concept of 'multi-targeting' is suggested for targeting closely related alien plant species as a group, after they have naturalised, but before they have become invasive. This is to challenge, where appropriate, the accepted paradigm in weed biocontrol, which targets individual species only after they have already become invasive and environmentally and/or economically harmful. The multi-targeting concept is tested in an experimental approach, complemented by a field survey. The experimental approach in Chapter 5 generated some insights about oviposition preferences of *R. conicus*, which are presented in Chapter 6. Chapter 6 has been accepted for publication (Groenteman *et al*., in press b), and is presented in its accepted version. Although Chapters 5 & 6 arise from a single experiment, the statistical analysis for Chapter 5 was done at a later date, after Chapter

6 had already been accepted for publication. In Chapter 5 I ended up using only a subset of the data, in contrast to Chapter 6, in which I used the full dataset. Therefore, some results may appear inconsistent between the two chapters. Chapter 5 is accompanied by an appendix (Appendix B), which reports some of the analyses on a larger subset. These results carry more resemblance to those reported in Chapter 6.

A unique population level approach to thistles is used in Chapter 7, where present population densities of multiple thistle species were measured for three regions of Canterbury, and compared to historical data from the mid 1980s levels, before two of the three biocontrol agents were released. It is asked whether thistle populations have changed in this time gap, and whether any such change could be related to biocontrol agents activity.

Finally, Chapter 8 presents a synthesis of this research and puts the main findings in context of research challenges in weed biocontrol (and thistle biocontrol in particular), in general, and in New Zealand.

Chapter 2[1]: Which species of the thistle biocontrol agent *Trichosirocalus* are present in New Zealand?

2.1 Introduction

The thistle biocontrol agent *Trichosirocalus horridus* (Panzer) was first introduced to New Zealand in 1984 and was released in the Canterbury plains of the South Island (Jessep, 1989b). By 1989, established populations were available for distribution to further sites in both the North and South Islands, and the weevil has been considered established in New Zealand ever since. The weevils introduced to New Zealand are originally from *Carduus nutans* L. from Neuenburg, Germany, and were established on *C. nutans* in Canada (Agriculture Canada, Regina Station, SK) prior to their introduction (Jessep, 1989a, b; Julien & Griffiths, 1998; P. Harris, pers. comm.).

Preliminary trials in New Zealand soon after the weevils establishment suggested it readily attacked the three *Carduus* spp. present in the South Island, *C. nutans*, *C. pycnocephalus* L. and *C. tenuiflorus* (the fourth *Carduus* sp., present only in New Zealand's North Island, *C. acanthoides* L., was not tested), and two *Cirsium* spp., *C. vulgare* and *C. palustre* (L.) Scopoli. It did not attack *C. arvense* (L.) Scopoli, *Silybum marianum* (L.) Gaertner, and *Onopordum acanthium* L. (Jessep, 1989b). The damage caused by weevil attack to both *Carduus* and *Cirsium* spp. in these trials was substantial.

In 1992 *T. horridus* from New Zealand was introduced to Australia for *C. nutans* control, and releases were initiated in 1993 (Woodburn, 1997). At the same time, scientists from CSIRO Australia were engaged in research in Europe towards a biocontrol programme for *Onopordum* spp. thistles (Briese *et al.*, 1994). One of the highly prioritized biocontrol agents was identified as *T. horridus*, partly because it had already been used successfully in Virginia for controlling the related *C. nutans* thistle (Kok, 1986, cited in Briese *et al.*, 1994). The weevils were introduced from Europe into quarantine in Australia, where it was noticed that they exhibited some consistent differences from the *T. horridus* introduced from New Zealand for *C. nutans* control (T. Woodburn, pers. comm.). Specimens were sent to a taxonomist (M. Alonso-Zarazaga), who re-described the species and revealed that, what was until then regarded as one species, *T. horridus*, was in fact a complex of three species (Alonso-Zarazaga & Sánchez-Ruiz, 2002). The samples sent from Australia included specimens of the *Onopordum* specialist from quarantine, and specimens from the population established on *C. nutans* near Canberra. The latter are considered progeny of the weevils

[1] This Chapter is now in press and may be cited as (Groenteman *et al.*, in press b).

introduced from New Zealand (Alonso-Zarazaga & Sánchez-Ruiz, 2002). The European samples from *Onopordum* in quarantine in Australia were identified as a new species, *T. briesei* Alonso-Zarazaga & Sánchez-Ruiz, with trophic linkage to the thistle genus *Onopordum* (larvae and adults did not survive on *Carduus* spp. and *Cirsium* spp.). Specimens from *C. nutans* from the Canberra region were also identified as a new species, *T. mortadelo* Alonso-Zarazaga & Sánchez-Ruiz, associated with thistles of the genus *Carduus*, mainly *C. nutans* (Alonso-Zarazaga & Sánchez-Ruiz, 2002). It was assumed that the weevils introduced from New Zealand to Australia were either *T. mortadelo* or a mixture of *T. mortadelo* and *T. horridus*. The species *T. horridus* was now associated with thistles of the genus *Cirsium* (Alonso-Zarazaga & Sánchez-Ruiz, 2002).

In Australia, efforts to establish weevils originating from New Zealand on *Cirsium* spp., were unsuccessful (A. Swirepick, pers. comm.), which settles with their identification as *T. mortadelo*, the *Carduus* specialist. In New Zealand, however, the weevils occur on both *Carduus* spp. and *Cirsium vulgare*, although their effect on *C. vulgare* populations is, for the most part, somewhat poor. *Trichosirocalus* spp. are extremely attractive biocontrol agents for weeds that exhibit a long-lived seed bank, such as *C. nutans* and *Onopordum* spp., as they reduce the plants vigour such that they either produce less seeds or even die prior to any seed production; and they also reduce plant biomass at the time that lapses until seed reserves in the soil are depleted (Briese, 2006). It was therefore desirable to find out whether the unsatisfactory control of *C. vulgare* in New Zealand was attributed to *T. horridus*, the *Cirsium* specialist, not being present here. In 2006, a survey was conducted in New Zealand to record which thistle species are attacked by which *Trichosirocalus* spp.

2.2 Methods and Materials

A field survey was initiated in autumn 2006 and lasted to the end of summer 2007. Regional Councils' staff visited release sites in different parts of the country, collected weevil specimens and, where possible, estimated whether thistle populations had decreased since the weevils' introduction into their region. All thistle species present at each site were noted, as well as the relative abundance of damaged rosettes (which can be readily distinguished by the black frass secreted by the feeding larvae). Where weevils were found on more than one thistle species at one site, they were collected separately for each thistle species. Sample size varied between sites, depending on the difficulty to obtain specimens. All weevils were preserved in 70% ethanol prior to identification.

For identification, all weevils were dissected under a stereo microscope. Their gender was recorded, and the adeagusof each male was examined against the key provided in

Alonso-Zarazaga and Sánchez-Ruiz (2002). All specimens were then mounted and deposited at the University of Canterbury arthropod collection for future referencing.

2.3 Results and Discussion

Different responses from Regional Councils in different parts of the country resulted in large variation in the number of samples collected per region. Eight regions (three in the North Island and five in the South Island, Figure 2.1) and 51 sites were sampled and 744 adults were dissected, 337 of which were males. In one region (Auckland), no weevils could be collected. All males in all samples were identified as true *T. horridus* (Table 2.1). Where estimated, *C. nutans* populations in New Zealand's South Island appear to have decreased since *T. horridus* introduction (Table 2.2).

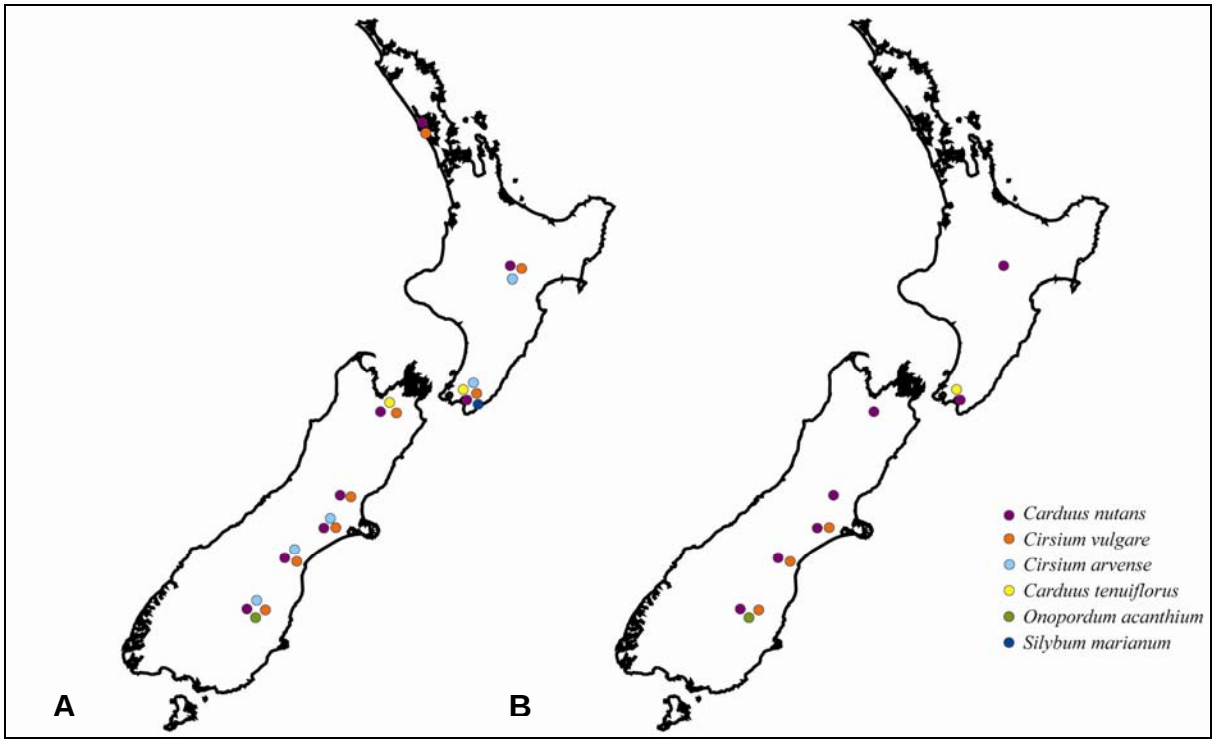

Figure 2.1: Regions of New Zealand visited in the survey with points marking: (**A**) thistle species present in each region and, (**B**) species from which weevils were collected.

Table 2.1: Geographic origin of *Trichosirocalus* spp. samples and species ID.

Region	Number of sites sampled	Host thistle sp.	Number of males examined	Species ID[1]
North Island				
Central NI	3	*C. nutans*	20	*T. horridus*
Greater Wellington	1	*C. nutans*	8	*T. horridus*
	1	*C. tenuiflorus*	6	*T. horridus*
South Island				
Tasman	1	*C. nutans*	20	*T. horridus*
North Canterbury	3	*C. nutans*	39	*T. horridus*
Mid Canterbury	2	*C. nutans*	19	
	2	*C. vulgare*	13	
South Canterbury	29	*C. nutans*	158	
	5	*C. vulgare*	12	*T. horridus*
Central Otago	1	*C. nutans*	15	*T. horridus*
	1	*C. vulgare*	5	*T. horridus*
	2	*O. acanthium*	22	*T. horridus*
Total	**44**[2]		**337**	***T. horridus***

[1]Based on male aedeagus; identified using the key provided in Alonso-Zarazaga and Sánchez-Ruiz (2002).
[2]In some sites weevils were collected from more than one thistle species.

Table 2.2: Estimates[1] of changes to *C. nutans* and *C. vulgare* populations since the introduction of *T. horridus*.

	Number of sites where populations were:		
	Estimated to have declined	Estimated to have not declined	Not estimated (unknown site history)
C. nutans			
North Island	3	3	0
South Island	16	6	17
C. vulgare			
North Island	1	1	3
South Island	2	2	18

[1]Subjectively estimated by Regional Council staff who collected weevils, or by land owners.

Table 2.3: Relative abundance of different thistle species in different regions of New Zealand, and fraction of sites in which *T. horridus* adults and/or damaged rosettes were abundant.

Region	Host species	Presence[1]	Relative abundance of thistle rosettes	Frequency of sites in which adult *T. horridus* and/or damaged rosettes were abundant[2]
North Island				
Auckland	*C. nutans*	1/1	moderately common	0/1
	C. vulgare	1/1	moderately common	0/1
Central NI	*C. nutans*	3/3	very common	1/3
	C. vulgare	2/3	moderately to very common	0/2
Greater Wellington	*C. nutans*	2/2	rare	1/2
	C. vulgare	2/2	rare	1/2
South Island				
Tasman	*C. nutans*	1/1	moderately common	0/1
	C. vulgare	1/1	rare	0/1
North Canterbury	*C. nutans*	3/3	moderately to very common	3/3
	C. vulgare	2/3	rare	0/2
Mid Canterbury	*C. nutans*	3/3	varies	1/3
	C. vulgare	3/3	moderately common	0/3
South Canterbury	*C. nutans*	30/30	moderately common mostly	21/30
	C. vulgare	14/30	rare to moderately common	5/14
Central Otago	*C. nutans*	2/3	moderately common	2/2
	C. vulgare	2/3	rare to moderately common	1/2
	O. acanthium	2/3	moderately to very common	2/2
NZ total	***C. nutans***	**45/46**		**29/45**
	C. vulgare	**27/46**		**7/27**

[1]Presence is expressed as the fraction of sites in which the thistle species was recorded, out of the total number of sites visited in each region.
[2]Rosette abundance was subjectively determined by Regional Council staff who collected weevils.

Three main points can be highlighted: (i) there is no evidence for any species other than *T. horridus* being present in New Zealand (Table 2.1); (ii) *T. horridus* readily attacks *Carduus*, *Cirsium* and *Onopordum* thistles (Tables 2.1 and 2.3); and (iii) *C. vulgare* appears to be, for the most part, not as strongly affected as *C. nutans* and *O. acanthium* by the weevil (Tables 2.2 and 2.3). The latter two points are inconsistent with the recent re-description of the species (Alonso-Zarazaga & Sánchez-Ruiz, 2002). According to this description, *T. horridus* should be able to feed on *Carduus* spp., but should show preference for *Cirsium* spp., in particular to *C. vulgare*. In addition, it has been indicated that *T. horridus* might be seen on *Onopordum* spp. in mixed thistle stands, but that it would not feed on it (M. Alonso-Zarazaga, pers. comm.).

The presence and feeding of *T. horridus* on *O. acanthium* in New Zealand is of special interest: in the early 1990s, not long after the weevil had been introduced to New Zealand and had established, T. Jessep (who was responsible for its introduction and distribution), realizing the weedy status of *Onopordum* spp. in Australia, decided to establish the weevil on

O. acanthium in New Zealand, as a measure to prevent this thistle from becoming weedy here (M. Turner, pers. comm.). *Onopordum acanthium* (the only *Onopordum* spp. present in New Zealand) is not common, and can mainly be found in Central Otago. A site was located in Central Otago, hosting an isolated dense *O. acanthium* stand, with no *C. nutans* to be found at a radius of 10 km. In 1993, 1,300 weevils, collected from *C. nutans*, were released at the site (M. Turner, pers. comm.). The weevils have established, and have dramatically reduced *O. acanthium* density at the site over several years. Today, only few thistles are germinating, fewer reach the flowering stage, and ones that do flower show reduced height and vigour (R. Groenteman, pers. obs.). Moreover, with the loss of prickliness attributed to the weevil larval feeding, the capitula appear palatable to the deer grazing on the farm, which further helps depleting the soil seed bank. The landowner assured us that he does not manage thistles on the farm by any other control means or land management practices. The weevils were never redistributed from that original site; yet we were able to collect them from another *O. acanthium* stand, about 50 km (aerial distance) away from the original site. At this distant site, part of the plants were high and vigorous, and exhibited no weevil feeding signs, whereas others appeared much less vigorous, exhibited feeding signs, and hosted the weevil (R. Groenteman, pers. obs.). It seems that the weevils dispersed by themselves to the site, and their impact is beginning to be noticeable. Adults that were collected from *O. acanthium* at both sites were identified, again, as *T. horridus*. At the self-introduced site, adult weevils were also collected from *C. vulgare*, and these, too, were identified as *T. horridus* (Table 2.1).

The results from the survey thus far present two paradoxes: firstly that *T. mortadelo* was collected from New Zealand and shipped to Australia but has not been found in our survey, and secondly, that *T. horridus* does not show a distinct preference for *Cirsium* thistles in New Zealand as reported elsewhere.

What, then, might the origin of *T. mortadelo* in Australia be? It appears that although CSIRO scientists have been engaged in searches for biocontrol agents in several European countries in the mid 1990s, the only *Trichosirocalus* sp. introduced into Australia as a result of this were the *Onopordum* specialist from Spain, later named *T. briesei*; and the only weevils released on *Carduus* thistles a few years earlier were of New Zealand origin (A. Sheppard, D. Briese, T. Woodburn, A. Swirepick, pers. comm.). If any *Trichosirocalus* spp. had been collected from *Carduus* thistles in Europe as part of these surveys they would have been examined in quarantine only, and not released (A. Sheppard, pers. comm.); whereas the *Carduus* weevils used in the revision were collected from established populations in Australia (D. Briese, pers. comm.).

It has been suggested that *T. mortadelo* may have disappeared from New Zealand or was out-competed by *T. horridus*. An examination of 30 specimens that were collected in South Canterbury in 1990, as part of a Lincoln University Master thesis, does not support this possibility. These specimens were collected at the original release site in New Zealand, where the weevils have been established the longest. This, most probably, was the site that sourced the weevils for the introduction to Australia. Sixteen of these specimens were males, and they were identified as *T. horridus*. Thus, it is hard to believe *T. mortadelo* was there at the time but was not represented in the sample.

A third paradox arises from the revision itself (Alonso-Zarazaga & Sánchez-Ruiz, 2002), and is supported by the personal communications surrounding this survey: that there is no evidence for *T. horridus* presence in Australia.

2.3.1 Conclusions and Outlook

To conclude, it appears that the *Trichosirocalus* spp. fauna in New Zealand consists of only one species, *T. horridus*, feeding on thistle hosts belonging to the genera *Carduus*, *Cirsium* and *Onopordum*. It is unclear why the weevils in New Zealand exhibit host utilization that is inconsistent with the recent revision. It also remains unclear where *T. mortadelo* in Australia originates from and why *T. horridus* is absent there. A similar survey in Australia would shed some light on these matters.

It is probably not desirable to re-introduce *T. horridus* from *C. vulgare* to achieve better control of this thistle, since the specialized populations may interbreed with the existing populations already well established on *Carduus*. However, it might be useful to harvest weevils from the few sites in New Zealand where they do well on *C. vulgare*, and redistribute those. It is certainly unjustified to introduce the *Carduus* specialist, *T. mortadelo*, since *T. horridus* is well established on *C. nutans* and appears to have a significant negative effect on this weed.

2.4 Addendum

Since this manuscript has gone into press, a few *T. horridus* from *C. nutans* in New Zealand have been examined by Dr. Rolf Oberprieler, a weevil specialist at the Australian National Arthropod Collection. The specimens from New Zealand were compared to two of the paratype specimens from Australia used by Alonso-Zarazaga and Sánchez-Ruiz for the description of the new species *T. mortadelo* (Alonso-Zarazaga & Sánchez-Ruiz, 2002). Dr. Oberprieler's conclusions could be summed up as follow:

1. The New Zealand specimens and the paratype specimens are the same species.

2. Neither follows the *T. mortadelo* description that is given in Alonso-Zarazaga and Sánchez-Ruiz's key (2002). All are more similar to *T. horridus*.
3. This resemblance does not reject nor confirm that *T. horridus* and *T. mortadelo* are two distinct species.
4. Without further study, we cannot confirm what species the New Zealand specimens (or the Australian paratype specimens) are.

This comparison resolved two of the paradoxes raised in this chapter – (i) that *T. mortadelo* was imported from New Zealand to Australia but was not found in my survey; and (ii) that there appeared to be no evidence for presence of *T. horridus* in Australia. It was now clarified that the weevils in New Zealand and the weevils in Australia which are considered descendants of weevils from New Zealand, all belong to a single species. However, the identity of this species, as well as the existence of the species complex remain unclear at this stage.

Following the presentation of this study at the XII International Symposium on Biological Control of Weeds in April 2007, the significance of clarifying the status of this species complex has been identified by the USDA. USDA Agricultural Research Service scientists and collaborators from Europe are planning to undertake morphological and genetic studies of *Trichosirocalus*. Specimens are currently being collected from the native range and from various parts of the introduced range (including New Zealand and Australia), and from various thistle hosts. Morphological and genetic features of these specimens will be compared in due course.

At this point, I cannot be certain of the identity of the weevils in New Zealand; I regard them hereafter as *T. horridus sensu* (Panzer).

Chapter 3: Abundance, phenology and impact of nodding thistle biocontrol agents on nodding thistle in New Zealand

3.1 Introduction

Nodding (musk) thistle, *Carduus nutans* (Asteraceae), a monocarpic herbaceous facultative-biennial, is a serious pasture weed in many parts of New Zealand (Delahunty, 1960; Jessep, 1989a). It reproduces by seeds only (Zwölfer & Harris, 1984; Popay & Medd, 1990), and is highly prolific (Feldman *et al.*, 1968; Kok, 1978; McCarty, 1982; Jessep, 1990; Popay & Medd, 1990).

A biocontrol programme against *C. nutans* in New Zealand commenced in the early 1970s. The first biocontrol agent, introduced in 1972, was the receptacle weevil, *Rhinocyllus conicus* (Coleoptera: Curculionidae), from *C. nutans* in Canada (Agriculture Canada, Regina Station), with those insects having been originally sourced from the Upper Rhine Valley in France (Jessep, 1989a). The weevil larvae develop by feeding on the receptacle tissue of *C. nutans* (and other thistles), which prevents seed development (Zwölfer & Harris, 1984). *Rhinocyllus conicus* is normally univoltine and can exhibit a partial second generation, if first generation larvae complete development when day length is 16 h or longer (Harris, 1984). *Rhinocyllus conicus* was reported to reduce early season viable seed production of *C. nutans* by 78% in Nebraska (McCarty & Lamp, 1982), however this figure is probably unreliable (Kelly & McCallum, 1995). *Rhinocyllus conicus* appeared to have controlled the weed in about six years from introduction in Virginia (Kok & Surles, 1975), with the estimated total seed loss of about 35% (Surles & Kok, 1978). Similar decrease in *C. nutans* density was recorded in Canada (Harris, 1984). A major contribution of *R. conicus* to successful *C. nutans* control was recently recorded in Oklahoma (Roduner *et al.*, 2003). In Australia, *R. conicus* is estimated to reduce *C. nutans* seed production by 7 - 36% (Woodburn, 1996a; Woodburn & Briese, 1996; Woodburn, 1997), whereas in New Zealand, it is estimated to reduce *C. nutans* seed production by about 36% (Kelly & McCallum, 1990, mean of % predation between 1982 to 1986), which is insufficient to bring *C. nutans* populations to decline in either country (Kelly & McCallum, 1995; Shea & Kelly, 1998; Shea *et al.*, 2005). In its native range in Europe, *R. conicus* is estimated to reduce *C. nutans* seed production by 35% (Sheppard *et al.*, 1994).

The crown-root feeding weevil *Trichosirocalus horridus sensu* (Panzer) (for clarification see Chapter 2) (Coleoptera: Curculionidae) was the second biocontrol agent introduced to New Zealand against *C. nutans*, in 1982, from Canada (Agriculture Canada, Regina Station), with those insects having been originally sourced from Germany. They had

become established in New Zealand by 1984 (Jessep, 1989a). *Trichosirocalus horridus* larvae consume meristem tissue in the crown-root zone of *C. nutans* rosettes. At high density, larval feeding can kill a plant; surviving plants suffer reduced performance and their foliage becomes spineless (Woodburn, 1997), which makes them more palatable to livestock (for an extended discussion on plant performance under sub-lethal *T. horridus* densities see Chapter 4). *Trichosirocalus horridus* is univoltine, but has an extended oviposition period in New Zealand (Jessep, 1989a). In Australia, *T. horridus* is estimated to reduce *C. nutans* seed potential (i.e., seed production per plant) by 72% (Woodburn, 1997). In Oklahoma, *C. nutans* densities decreased faster at sites with *T. horridus* (and *R. conicus*) than in sites with no *T. horridus* (and only *R. conicus*) (Roduner *et al.*, 2003) and in Virginia, *T. horridus* has successfully reduced plumeless thistle, *Carduus acanthoides* densities at sites with little or no *C. nutans* (Kok & Mays, 1991). It has to be noted, however, in light of the uncertainty surrounding the taxonomic identity of *T. horridus* that, while the weevils in New Zealand and Australia have been confirmed to belong to the same species, the weevils in North America may or may not be of the same species. While the incidence of attack by *T. horridus* was lower on small *Carduus thoermeri* Weimann (a synonym of *C. nutans* ssp. *leiophyllus* (Petrovič) Stoj. & Stef.) and *C. acanthoides* plants than on larger plants in Virginia (Sieburth & Kok, 1982), both large and small *Onopordum nervosum* Boiss. thistles were attacked by *Trichosirocalus briesei* in Australia (Briese *et al.*, 2002). It was interesting to test the pattern of natural abundance of *T. horridus* in relation to the size of the resource - *C. nutans* rosette in New Zealand, bearing in mind it may not be the same species studied in North America.

The third *C. nutans* biocontrol agent, the gall-forming fly *Urophora solstitalis* (Diptera: Tephritidae), was introduced in 1992 from a French biotype that was first established in Australia (Hayes, 2007). Larvae induce the formation of woody galls in the receptacle of *C. nutans* capitula, which not only prevent seed development within the attacked capitulum, but are also strong sinks for nutrients from the entire plant (Harris, 1989a). *Urophora solstitialis* is bivoltine; its first generation coincides with *R. conicus*, and its second generation occurs in the absence of *R. conicus*, when the majority of late *C. nutans* capitula are produced. In Australia, a 45% reduction in seed set was achieved in the first year after *U. solstitialis* introduction (Woodburn, 1996b; Woodburn & Cullen, 1996), and the later 70% reduction of *C. nutans* seed rain is attributed largely to *U. solstitialis* (Woodburn & Briese, 1996). In its native range in Europe, where a large number of natural enemies of *C. nutans* and of *U. solstitialis* are present, it was estimated to account for only 15% seed reduction (Sheppard *et al.*, 1994). It has been suggested that *U. solstitialis* may be outcompeted by *R.*

conicus when they occur together (Harris, 1989a), and this appears to be the case in the first generation of *U. solstitialis* in Australia (Woodburn, 1996a).

Establishment in New Zealand of all three biocontrol agents is well documented (Jessep, 1975, 1989b; Harman *et al.*, 1996; Hayes, 2007). However, impact on the target weed, *C. nutans*, has been studied for only *R. conicus* (Popay *et al.*, 1984; Kelly & McCallum, 1990; Kelly *et al.*, 1990; Kelly & Wood, 1991; Kelly & McCallum, 1995; Shea & Kelly, 1998).

In order to address this gap in knowledge on *C. nutans* biocontrol agents in New Zealand, the current chapter sets three objectives: to (a) measure the abundance of *T. horridus* larval stages in rosettes in Canterbury, (b) follow the phenology of *R. conicus* and *U. solstitialis*, and (c) measure the impact of *R. conicus* and *U. solstitialis* on *C. nutans* seed production. To achieve these objectives, field surveys and an experiment were incorporated.

3.2 Methods

3.2.1 *T. horridus* abundance in *C. nutans* rosettes

The pattern of *T. horridus* attack on *C. nutans* was surveyed in North, Mid and South Canterbury. Three sites per region were haphazardly selected, in which *C. nutans* rosettes were sampled twice, in winter 2005 and winter 2006. Sampling during winter represents peak levels of larval infestation, and avoids effect of rosette mortality attributed to *T. horridus*, which occurs in late spring. The sites were: North Canterbury (Culverden area): Isolated Hill Rd. (IHR), 42°45.24’ S 172°56.85’ E; Leaches Rd. (LR), 42°43.51’ S 172°56.45’ E; The Mound Rd. (MR), 42°47.09’ S 172°55.62’ E; Mid Canterbury (Rakaia Gorge area): Coalgate (CO), 43°28.70’ S 172°00.01’ E; Homebush (HB), 43°28.09’ S 172°01.21’ E; Hororata (HO), 43°32.89’ S 171°55.66’ E; and South Canterbury (Ashburton area): Hamptons Rd. (HR), 43°56.41’ S 171°57.16’ E; Jamisons Rd. (JR), 43°45.19’ S 171°49.58’ E; Windermere Rd. (WR), 43°57.80’ S 171°35.71’ E. At each site five points were randomly selected (by dividing each paddock to an imaginary grid and computer-generating random coordinates) at which the six nearest *C. nutans* rosettes within a radius of 3 m were dug up, their longest leaf length was measured in cm, and the root crown taken to the lab. If not enough rosettes could be collected at a certain point, more points were randomly selected, until a minimum of 30 rosettes per site was reached. Work in Virginia found that larger rosettes were significantly more prone to attack by *T. horridus* (Sieburth & Kok, 1982). Therefore, to assure sufficient sample size, additional large rosettes (leaf length longer than 20 cm) were taken if fewer than three of the six nearest rosettes at each point had a longest leaf length of less than 20 cm, to a total of up

to three per point. Thus, altogether a site had a minimum of 30 and a maximum of 45 rosettes collected, depending on the size distribution. In the lab, the crown-root diameter was measured in mm, and *T. horridus* larvae counted and their instar recorded on at least 20 of the rosettes per site (except in three cases where only 18, 13 and 17 crowns were processed per site; Table 3.1). Leaf lengths and crown-root diameters were divided to three to four categories. Categories were deemed a more informative way of presentation than continuous variables if on-site estimates are ever to be attempted using these relationships. This is because size categories can be fairly easily estimated, whereas continuous linear relationships would require actual measurements before any useful inferences could be made. This is especially true for leaf length categories which do not even require any destructive action to estimate. The distributions of attack (number of larvae of all instars per rosette) per leaf length category and per crown-root diameter category are presented. The effects leaf length and crown-root diameter on the number of larvae per unit area of crown-root were analysed in mixed effects models with site and year as grouping factors.

Table 3.1: The number of crowns processed for each site (coded as in the text above) in 2005 and 2006.

	North Canterbury			Mid Canterbury			South Canterbury		
	IHR	LR	MR	CO	HB	HO	HR	JR	WR
2005	32	23	23	43	25	26	46	29	18
2006	13	20	20	17	20	20	20	23	20

3.2.2 Seed predators effect on *C. nutans*

Field experiment

Carduus nutans plants were grown in the University of Canterbury glasshouses from seeds collected in summer 2005 around Canterbury. In late autumn 2005, 120 plants were transplanted as small rosettes to the experimental plot, an uncultivated grassy area that was regularly mown, at the Landcare Research Lincoln campus, as part of the multi targeting experiment (Chapter 5). *Carduus nutans* plants were arranged in 12 plots (ten plants per plot) within six blocks (the complete experimental design is given in detail in Chapter 5). The experiment site was naturally colonised by *R. conicus* and *U. solstitialis*; no controlled releases of biocontrol agents were made. The number of capitula was counted on each plant when capitula were harvested. The plants were checked for ripening capitula frequently (every two to ten days, depending on ripening rate of capitula. When each capitulum was collected its height, position (primary, secondary or tertiary) and external diameter were recorded. The frequent collection did not allow for any seed dispersal or predation by

goldfinches. Capitula were stored to allow time for seed predator development, after which their receptacle diameter was measured and any signs of attack by *R. conicus* and *U. solstitialis* were recorded. *Rhinocyllus conicus* attack signs included egg cases (recorded as 0 – none, 1 - low, 2 - medium and 3 - high egg density), larvae, pupae, adults and typical feeding damage. *Urophora solstitalis* attack signs included larvae, pupae, adults and excess empty galls. The level of feeding damage in attacked capitula was ranked from 0 to 3 (0 – none, 1 – little, 2 – medium and 3 – high damage), and viable seeds were counted for most capitula. The first capitulum ripened on 19 December 2005, and collections continued until 16 May 2006. About two thirds of the 90 plants that bolted successfully were harvested in full during this time, and the remainder plants for which not all capitula ripened by 16 May were destroyed.

The number of viable seeds from unattacked capitula was regressed against capitulum external diameter (in mm), accounting for non-independence of capitula within plants. The analysis showed a poor fit to the data, with some capitula showing increasing seed number with increasing diameter, while others showed low seed number across a range of capitulum size (Results, Figure 3.3). It was suspected that capitula flowering later in the season may be less successfully pollinated (due to poorer pollinators' activity when temperatures drop). Otherwise, capitula at secondary and tertiary positions may suffer from depleted plant resources, which may result in lower seed-set. The effects of time in the season and capitulum position on seed production were therefore tested in a mixed effects model, using plants, plots and blocks as grouping factors. However, as not all plants and all plots had ripened capitula at all combinations of times and positions, the model ignored these grouping factors, and only used blocks as a grouping factor. This analysis showed that neither time in the season, capitulum position or their interaction allowed good fits of seed number vs. capitulum area (Table 3.4). I therefore did not use these data to further estimate seed output per capitulum. Instead, in the absence of further testable factors for the poor fit of my data, I used the formula calculated by Popay *et al.* (1984), which shows high correlation and which agrees with other formulae for nodding thistle in New Zealand and Australia (Kelly & McCallum, 1990):

Seed output = (receptacle area in $mm^2 \times 0.76$) + 76.8 ($r^2 = 0.83$)

To estimate seeds destroyed per seed predator, seed output was calculated for capitula damaged by seed predators, using the Popay *et al.* (1984) formula; the actual seed counted from these capitula were subtracted from this estimate (no negative values were allowed), and

the new estimate, divided by the number of seed predators present in each capitulum, was averaged for each seed predator. Capitula attacked by both seed predators were not included in producing these estimates. Early larval instars of the seed predators were excluded because their consumption is negligible in comparison to later instars. To account for variation in seed destruction, the effects of larval density, capitulum position and time within the season were explored.

Seed loss per plant was estimated for plants that had at least 80% of their capitula harvested and processed (58 plants out of 90 plants that produced capitula). For each of these plants, potential seed output was estimated in the absence of seed predation: actual seed counts for unattacked capitula were combined with Popay *et al.*'s (1984) formula estimates for attacked capitula (and for unattacked capitula that did not have their seed counted), to estimate seed output per plant in the absence of seed predation. Seed output after seed predation was estimated for each of these plants, using the same procedure as before for unattacked capitula, but using actual seed counts for damaged capitula if such counts were done, or estimating seed loss for damaged capitula that had not had their seeds counted from the predation estimates calculated above. The estimate of seed production, accounting for predation, was calculated for each seed predator separately, and for both seed predators together. The proportion of seed loss was calculated using the quotient of these two estimates.

Field survey

During the time when the above experiment was running, one site that was used in the *T. horridus* survey, the Hamptons Rd. site in South Canterbury, was followed for seed predator attack: from 20 December 2005, one day after the first ripe capitulum was collected at the experiment above, the site was visited approximately once a fortnight. The number of flowering plants and the number of flowering capitula (capitula with at least one floret open) were recorded, and all ripe capitula were collected, from a plot of 34 m length by 1 m width. In the lab, these capitula were measured (external diameter and receptacle diameter), and the presence, number, and instars of larval *R. conicus* and *U. solstitialis* recorded. Sampling ceased in mid autumn, when very few plants were still flowering, but no seed predators were found in two consecutive samples. This prompted the decision to stop collections from the experiment above soon afterwards. The survey was repeated in 2006-07, but during this season the thistles in this site were mowed three times, which would have killed any seed predators and eliminated any second generation *U. solstitialis*. Only data from 2005-06 were therefore used.

Seed predation per larval *R. conicus* and *U. solstitialis* calculated from the experiment above were used to estimate seed loss in the field population: seed potential of collected

capitula was estimated from the receptacle diameter, using the relationship established by Popay *et al.* (1984). The number of seed lost per capitulum to either seed predator on each collection date was estimated from the number of larvae recorded, using the estimates of seed predation per larva calculated from the experiment above. A proportion of seed loss was then estimated for each sampling date by calculating the fraction of flowering capitula present on site on that date of the total flowering capitula counted throughout the season, and multiplying by the proportion of seed loss estimated for that date from the processed capitula. Finally, the latter proportions were summed to give a seasonal estimate of seed loss to either seed predator and for both. These calculations were done for the 2005-06 season only.

3.3 Results

3.3.1 *T. horridus* abundance in *C. nutans* rosettes

In both 2005 and 2006, a trend of increased *T. horridus* attack with increased rosette size (expressed as either longest leaf length or as crown diameter) was apparent (Figure 3.1). In most cases, the maximum number of larvae found in a single rosette exceeded 100, and in all regions and years more than half the rosettes had larvae (Table 3.2). Larger rosettes hosted significantly more larvae per mm^2 crown-root (Table 3.3).

Table 3.2: Maximum number of *T. horridus* larvae found in a single *C. nutans* rosette and percentage of rosettes with larvae in three regions of Canterbury over two seasons

	North Canterbury		Mid Canterbury		South Canterbury	
	No. larvae	% attacked	No. larvae	% attacked	No. larvae	% attacked
2005	119	72	199	55	253	80
2006	130	85	129	61	73	57

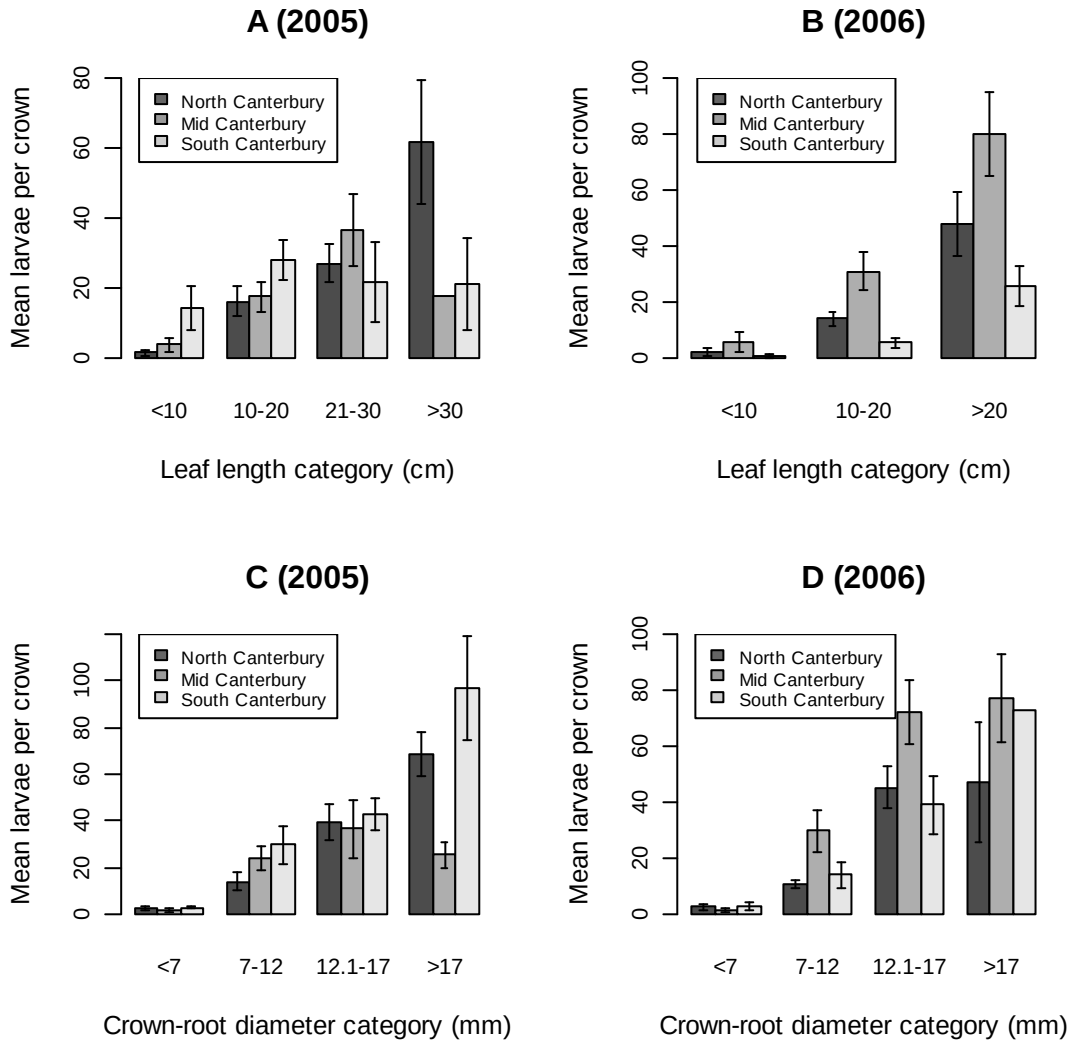

Figure 3.1: Mean *T. horridus* larvae (all instars) per *C. nutans* crown at three to four leaf length categories (A & B) and at four crown-root diameter categories (C & D) for three regions of Canterbury in 2005 (A & C) in 2006 (B & D).

Table 3.3: Number of *T. horridus* larvae (log transformed) per mm^2 crown-root **(a)** at increasing leaf length and **(b)** at increasing crown-root diameter: generalised linear mixed effects model with Poisson distributions and with site and year as grouping factors.
(a) Based on 407 crown-roots from 9 sites and two years.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-2.334	0.272	-8.591	<0.001
Longest leaf length	1	0.036	0.013	2.841	0.005

(b) Based on 404 crown-roots from 9 sites and two years

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-2.085	0.246	-8.463	<0.001
Crown diameter	1	0.036	0.019	1.888	0.059

3.3.2 Seed predators effect on *C. nutans*

The degree of similarity between the questions asked in the field experiment and in the field survey calls for the two datasets to be presented simultaneously.

The relationship between capitulum area and seed count in *C. nutans* in the experiment exhibited two cohorts: one in which seed production increased linearly with increasing capitulum area, and another, in which seed production was low and unrelated to capitulum area (Figure 3.2). The linear relationship for the entire sample is (after accounting for non-independence of capitula within plants):

Seeds per capitulum = 10.07 + 1.12 × external diameter (mm)
Based on 1975 capitula from 86 plants.

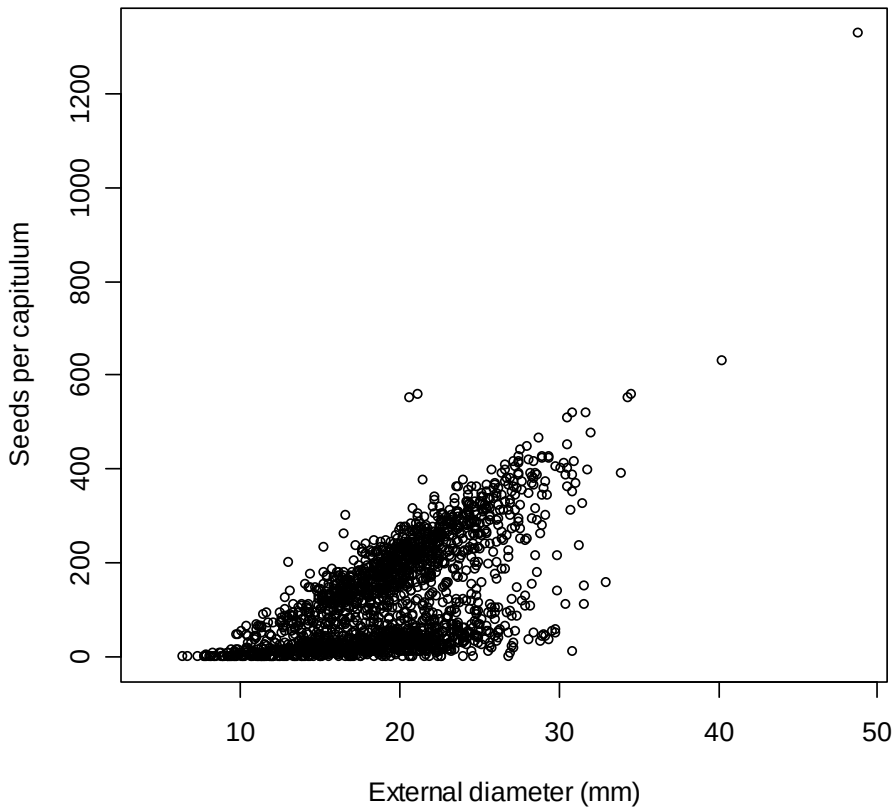

Figure 3.2: Relationship between *C. nutans* capitulum area (calculated from external diameter) and seeds produced per capitulum at the experimental site at Landcare Research Lincoln campus.

An analysis of seed production per capitulum partitioned to capitulum position and time in the season (Figure 3.3, Table 3.4) did not reveal a pattern that could accurately predict the relationship between capitulum area and seed production. Therefore, where seed estimates were required for *C. nutans* in this chapter and in the following

chapters, the formula based on receptacle area produced by Popay *et al.* (1984) was used.

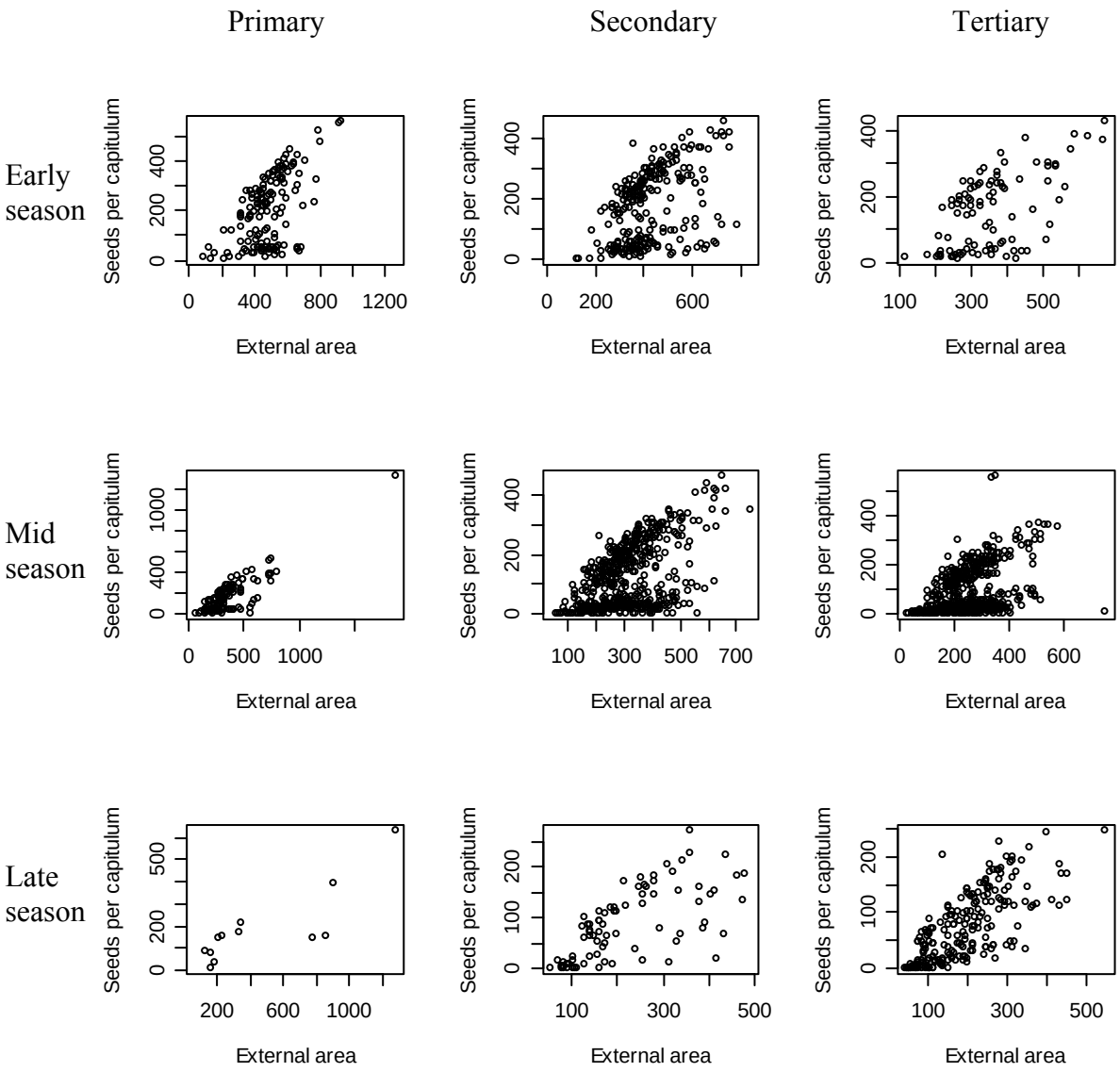

Figure 3.3: Relationship between *C. nutans* capitulum area (calculated from external diameter) and seeds produced per capitulum at the experimental site at Landcare Research Lincoln campus, partitioned to combinations of position and time in the season.

Table 3.4: The effect of capitulum diameter, capitulum position, and time in the season on *C. nutans* measured seed production: generalized linear mixed effects model with a Poisson distribution and with block as a grouping factor.
Based on 1973 capitula from 6 blocks[1].

Fixed effects					
	Df	Estimate	Std. Error	z value	*P*
Intercept[2]	1	4.734	0.038	123.40	<0.001
External diameter	1	0.566	0.002	231.25	<0.001
Secondary position	1	0.074	0.006	12.11	<0.001
Tertiary position	1	0.030	0.007	3.99	<0.001
Mid season	1	-0.028	0.005	-5.70	<0.001
Late season	1	-0.110	0.009	-12.56	<0.001

[1]For detailed experimental design see Chapter 5
[2]Intercept: primary position, early season

The flowering status of the site in South Canterbury during the time of the experiment is presented in Table 3.5. Both 2005-06 and 2006-07 are presented, but 2006-07 is not discussed any further. The proportion of capitula attacked by either seed predator reveals a similar pattern in the field survey (2005-06) and the experiment (Figures 3.4 & 3.5 respectively; see also Table 3.6 for the experiment). They portray a single generation of *R. conicus* attacking early in the season, a low peak of first generation *U. solstitialis* 6-8 weeks later, which overlaps with *R. conicus*, and a second generation of *U. solstitialis* about three months into the season. At the experimental site, a partial second generation of *R. conicus* appeared to attack at about the same time that *U. solstitialis* second generation started to appear. This second generation of weevils probably originated from the annual, early flowering species, *Carduus tenuiflorus* and *Carduus pycnocephalus*, used in the multi targeting experiment (see Chapter 5); most infested capitula from the multi targeting experiment were collected, but it is likely that some dropped off the plants and escaped collection. Despite the similar pattern, attack by *R. conicus* and by first generation *U. solstitialis* at the field site in South Canterbury never reached similar levels as at the experimental site (raw mean ± SE percentage of attacked capitula reached 38.0% ± 5.9 in the experiment and only 19.4% ± 5.0 in the field survey); however, my observation is that the biocontrol agents are abundant in South Canterbury, and I suspect that 19.4% attack at the survey site may under-represent the magnitude of attack in the region.

The partitioning of attacked capitula to positions (Figures 3.6 & 3.7) reveals that most primary capitula are attacked. Also, anecdotally, it appears that when both seed predators are present simultaneously, *R. conicus* utilizes more primary capitula than *U. solstitialis*, however, further testing is required to varify this pattern. Nonetheless, despite the fewer

capitula attacked by *U. solstitialis* in the first days of its appearance, it maintains a high proportion of its attack on primary capitula, (Figure 3.6).

Each *R. conicus* larva was estimated to destroy 34.3 ± 2.2 seeds, while each *U. solstitialis* larva was estimated to destroy 21.3 ± 1 seeds. Partitioning of seed destruction by larval density shows very high destruction per larva in low densities (1-4 larvae per capitulum) compared to densities of five or more, in both *U. solstitialis* and *R. conicus* (Figure 3.8). Capitulum position and time in the season do not account for much variation in seed destruction (Figure 3.9). It has to be noted that in *R. conicus*, any late season attack resulted in only low densities (and hence apparent higher seed destruction per larvae). Per-plant seed loss to *U. solstitialis* reached similar estimates in both the experiment and the field survey, while seed loss to *R. conicus* appeared higher at the experimental site (Table 3.7); this settles with the overall lower attack level associated with *R. conicus* at the field survey in comparison to the attack level at the experiment as is evident from Figures 3.4 & 3.5.

Table 3.5: *C. nutans* flowering status at the site on Hamptons Rd., South Canterbury in (**a**) 2005-06 and in (**b**) 2006-07.

(a). 2005-06

Date	20.12	03.01	18.01	04.02	15.02	28.02	14.03	26.03	10.04	24.04	08.05
Days after 20 December 2005	0	14	29	46	57	70	84	96	111	125	139
Flowering plants	26	43	53	50	41	27	25	26	21	15	11
Flowering capitula	65	142	211	244	126	76	43	56	59	38	20
Capitula collected (ripe)	19	68	90	151	51	51	14	20	26	22	7
Capitula with *R. conicus*	6	22	13	8	1	1	0	0	0	0	0
Capitula with *U. solstitialis*	0	0	6	16	1	0	7	6	7	0	0
Capitula with any seed predator	6	22	19	23	2	1	7	6	7	0	0

(b). 2006-07

Date	12.12	27.12	09.01	24.01	07.02	21.02	08.03	21.03
Days after 12 December 2006	0	15	28	43	57	71	86	99
Flowering plants	0	191	181	230	286	262	76	85
Flowering capitula	0	7	59	120	529	582	97	61
Capitula collected (ripe)	0	0	1	28	94	245	30	26
Capitula with *R. conicus*	0	0	1	3	10	13	4	2
Capitula with *U. solstitialis*	0	0	0	3	0	11	3	1
Capitula with any seed predator	0	0	1	6	10	22	7	3

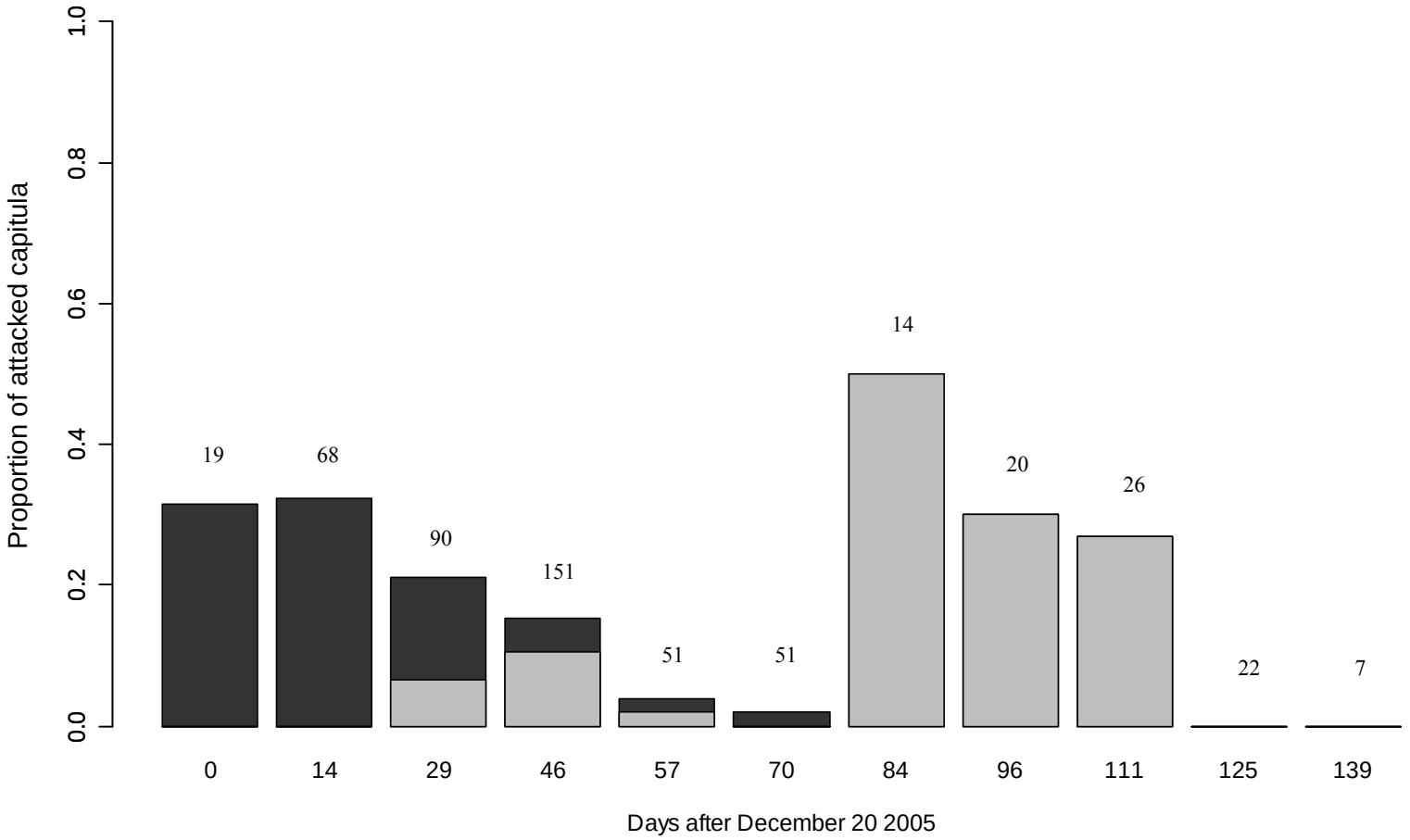

Figure 3.4: Proportion of *C. nutans* capitula attacked by *R. conicus* (dark), and by *U. solstitialis* (light) at a site in South Canterbury, between 20 December 2005 and 8 May 2006. All ripe capitula were collected from a plot of 34 m x 1 m approximately once a fortnight. The number of capitula collected on each occasion is presented above each bar.

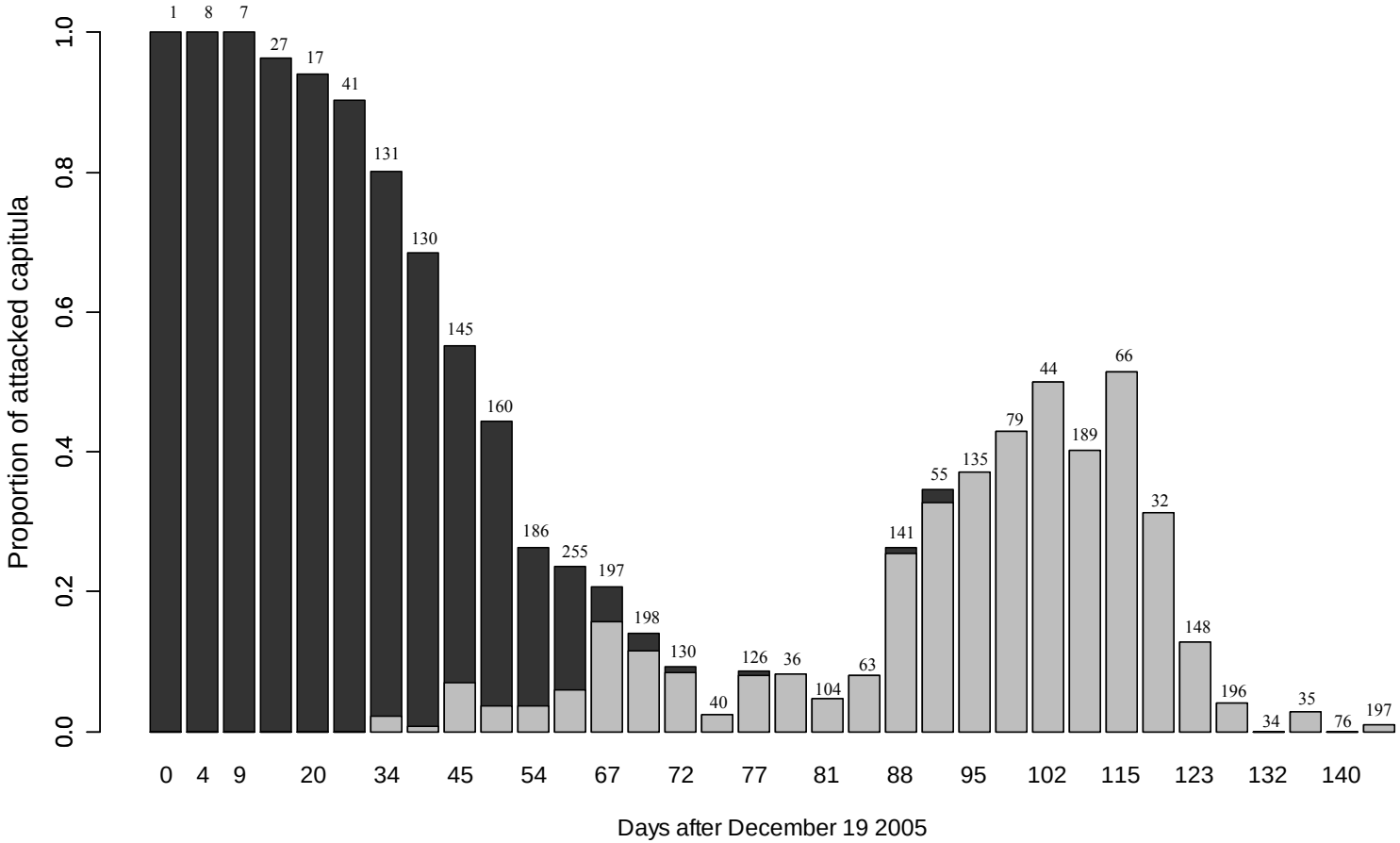

Figure 3.5: Proportion of *C. nutans* capitula attacked by *R. conicus* (dark), and by *U. solstitialis* (light) at the experimental site at Landcare Research Lincoln campus, between 19 December 2005 and 16 May 2006. All ripe capitula were collected. The number of capitula collected on each occasion is presented above each bar.

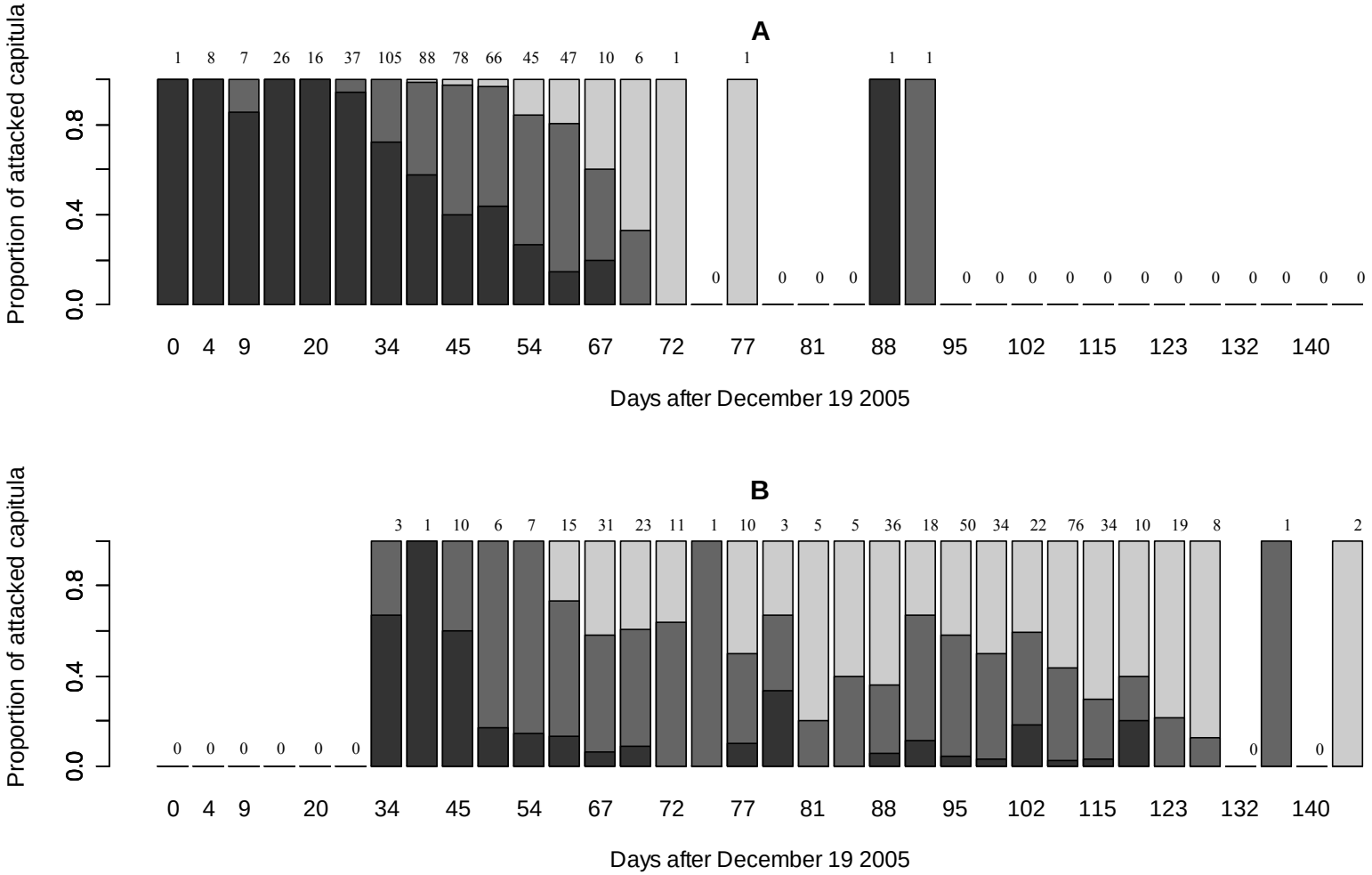

Figure 3.6: For attacked *C. nutans* capitula, the partitioning to positions on the plants (primary – dark gray; secondary – mid gray; tertiary – light gray) of (**A**) *R.conicus* attack and (**B**) *U. solstitialis* attack. The number of attacked capitula is presented above each bar.

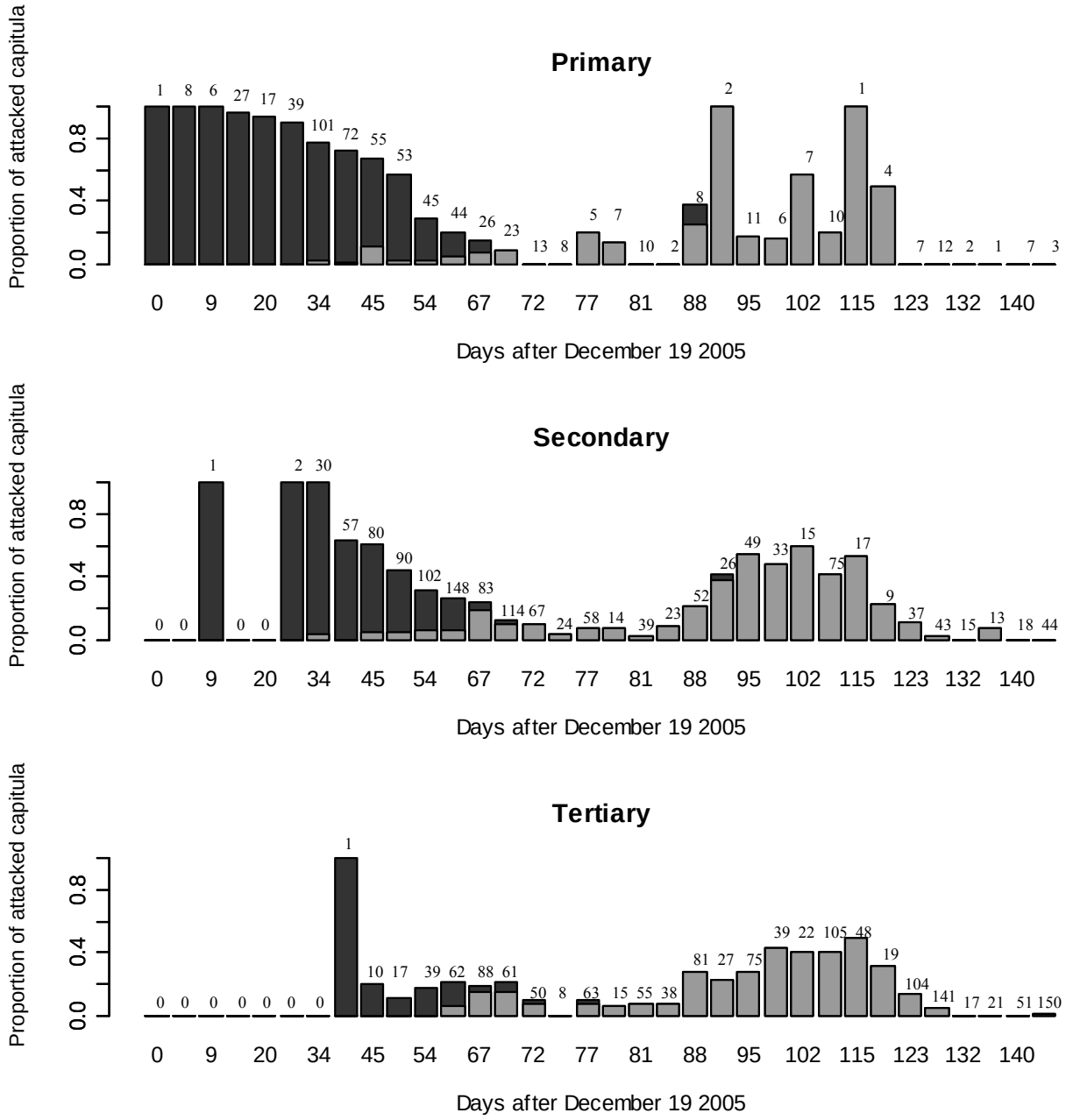

Figure 3.7: Proportion of *C. nutans* capitula attacked by *R. conicus* (dark) and by *U. solstitialis* (light) on each collection date, out of those that ripened on that date, at the experimental site at Landcare Research Lincoln campus, partitioned to positions. The number of ripe capitula of each combination of position and date is presented above each bar.

Table 3.6: Median capitulum height and external diameter for *C. nutans* capitula which ripened on each collection date, partitioned to positions, and the number that were attacked by either seed predator, or by both

		Median capitulum height (mm)			Median external capitulum diameter (mm)				
Date	Days after 19 Dec. 2005	Primary	Secondary	Tertiary	Primary	Secondary	Tertiary	Number attacked by either seed predator	Number attacked by both seed predators[1]
19.12	0	844			38.10			1	0
23.12	4	655			29.06			8	0
28.12	9	662	753		30.11	25.52		7	0
02.01	14	750			27.50			26	0
08.01	20	605			31.28			16	0
18.01	25	839	1037		27.94	27.57		37	0
22.01	34	677	879		26.18	24.41		105	3 (2, 1, 0)
27.01	39	643	859	635	25.97	24.53	19.24	89	0
02.02	45	645	815	820	24.67	23.73	19.69	80	8 (4, 4, 0)
07.02	50	684	851	1040	24.19	22.90	21.12	71	1 (1, 0, 0)
11.02	54	560	783	980	24.85	23.09	20.56	49	3 (1, 2, 0)
17.02	60	529	753	1007	23.94	22.08	20.05	60	2 (1, 0, 1)
24.02	67	596	760	890	22.28	21.62	18.70	41	0
27.02	70	455	650	990	20.25	20.53	20.13	28	1 (0, 1, 0)
01.03	72	512	680	858	21.22	20.15	18.80	12	0
03.03	74	425	530	661	22.38	20.42	19.41	1	0
06.03	77	607	617	765	20.24	20.30	17.50	11	0
08.03	79	345	645	1013	19.97	21.92	21.32	3	0
10.03	81	468	550	760	19.76	18.63	16.61	5	0
13.03	84	319	590	750	20.69	18.57	16.95	5	0
17.03	88	411	503	790	19.20	19.22	18.90	37	0
20.03	91	397	571	620	18.73	18.92	18.44	19	0
24.03	95	332	574	790	19.89	18.65	16.88	50	0
27.03	98	353	530	663	19.42	18.81	18.13	34	0
31.03	102	283	546	675	17.81	17.91	17.57	22	0
05.04	107	296	460	674	17.12	18.07	16.95	76	0
13.04	115	376	546	760	10.60	19.02	17.46	34	0
18.04	120	454	450	670	19.03	13.64	13.78	10	0
21.04	123	290	430	632	16.80	15.67	15.94	19	0
26.04	128	379	435	596	19.14	16.75	15.74	8	0
01.05	132	283	440	400	14.62	14.93	13.91	0	0
05.05	136	286	326	515	17.25	16.65	14.94	1	0
09.05	140	567	491	595	31.57	12.84	11.16	0	0
16.05	148	316	368	494	14.44	12.92	12.81	2	0
	Total							967	18

[1]Numbers in parentheses in cells that had capitula attacked by both seed predators are the partitions of these capitula to primary, secondary and tertiary positions, respectively. (The two primary capitula collected on 22 January, 2006 only had external signs of attack by *R. conicus* (namely, eggs); internally, only *U. solstitialis* signs were recorded).

Table 3.7: Seed production per *C. nutans* plant at the experimental site at Landcare Research at Lincoln (raw means ±SE), and estimates of proportional seed loss to the seed predators at the experimental site, and at the field survey site at Hamptons Rd. in South Canterbury

		Capitula production per plant	Seed production per plant	Seed loss to *R. conicus* (%)	Seed loss to *U. solstitialis* (%)	Seed loss to both (%)
Experiment	Mean	47.5 (±6.3)	6873 (±1097)	14.3 (±1.7)	8.8 (±1.2)	22.8 (±1.9)
Field survey 2005	Mean			4.2	7.2	11.3

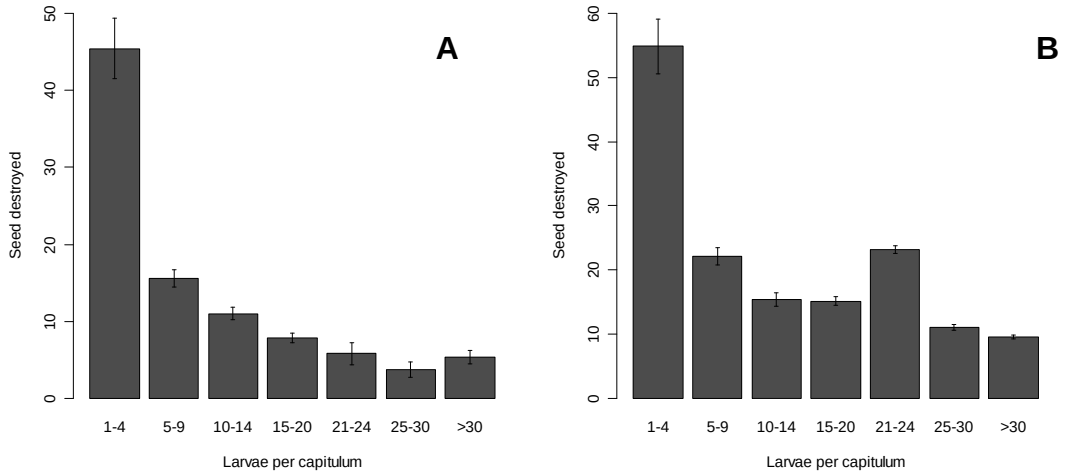

Figure 3.8: Seed destruction per larva of **(A)** *U. solstitialis* and **(B)** *R. conicus* at increasing larval densities.

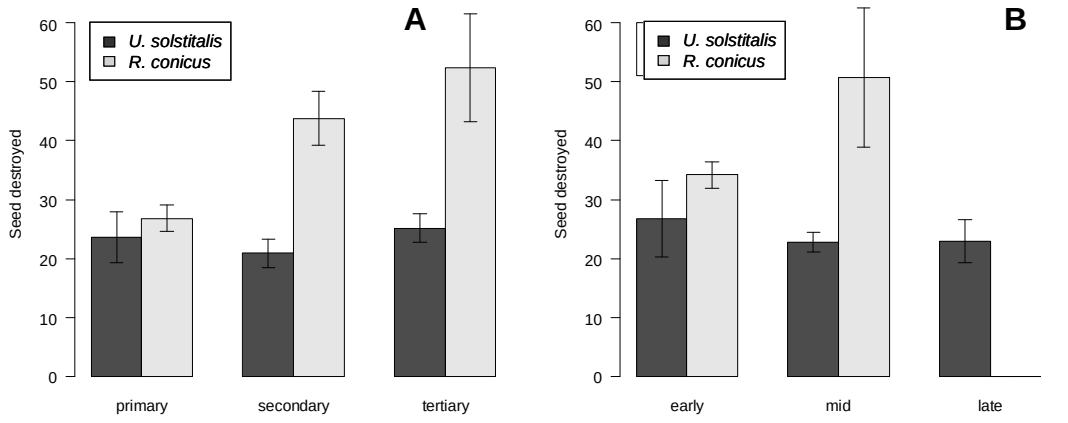

Figure 3.9: Seed destruction per larva of *U. solstitialis* (dark bars) and *R. conicus* (light bars) at **(A)** different capitula positions and **(B)** different times in the growing season.

3.4 Discussion

Populations of facultative-biennial weeds represent multiple cohorts of germination with great variability of sizes, and their biocontrol can greatly benefit from root-feeding agents that distribute attack over various sizes (Briese *et al.*, 2002). In my survey *T. horridus* larvae were found in rosettes of all size categories (Figure 3.1). In many cases, however, especially at early stages of infestation, attack signs could not be detected without destruction of the crown (pers. obs.); this may explain why the Virginian study (Sieburth & Kok, 1982), which only used nondestructive means, failed to detect attack in smaller rosettes. As in the Australian observations, more *T. horridus* larvae were found in larger rosettes in Canterbury (Figure 3.1). This trend was clear for crown diameter as well as for leaf length, in both 2005 and 2006, and in all three regions, though different larval densities were apparent in the different regions. These regional differences in larval densities may be related to differences in thistle densities in different regions (see Chapter 7 for differences in *C. nutans* population densities in regions of Canterbury). Briese *et al.* (2002) have shown that increased densities of *T. briesei* resulted in a similar rate of increase of attack on both small and large *Onopordum* spp. rosettes; this suggests that, provided agent populations are high enough, all plants could be attacked at a damaging level. They suggested higher attack on larger plants probably does not indicate greater attraction to larger plants, but rather the higher frequency of encounters with larger plants, and a tendency of females to remain longer on larger plants, hence ovipositing more; Gerber *et al.* (2007a) add (in a similar context) that larger plants may simply exhibit higher numbers of suitable oviposition sites. Larger plants in my study hosted significantly more *T. horridus* larvae per unit area of crown-root (Table 3.3), which indeed demonstrates higher rate of attack on larger plants. Higher attack on larger plants has also been documented in other root feeders (Prins *et al.*, 1992; Pomerinke *et al.*, 1995; Gerber *et al.*, 2007a).

Briese *et al.* (2002) also found a weak negative relationship between the number of *T. briesei* larvae and plant density: *O. nervosum* plants in denser patches were slightly less likely to be attacked. On the local scale, this indicates the weevils are well capable of locating isolated plants, and, if these are attacked more frequently than plants in dense patches, this means the weevil could modify the level of aggregation of the weed, and hence its impact (Briese *et al.*, 2002).

The numbers of *T. horridus* larvae found per crown are comparable to field densities found in Kansas: 7 to 34 larvae per plant on average, ranging between 0 and 77 larvae per individual plant (Milbrath & Nechols, 2004b). Milbrath and Nechols (2004b) also found that densities of less than 20 larvae per plant caused no more than a change in plant architecture, and did not affect *C. nutans* capitulum or seed production. Rosettes in that experiment ranged

from 15 to 30 cm in diameter (comparable to just over 7.5 to 15 cm longest leaf length). Experimental larval densities in Kansas were consistently lower than the numbers inoculated (Milbrath & Nechols, 2004b, a). Their information, combined with the field infestation recorded in my study, was used in selecting numbers to generate sub-lethal infestation in the experiment in Section 4.2 of this thesis.

Carduus nutans is a highly prolific seed producer. Estimates of average seed production in New Zealand include, about 6,200 viable seeds per plant (Jessep, 1990), about 8,600 seeds per m^2 with about 65% viability from 29 capitula per m^2 (Popay *et al.*, 1984), and the comparable estimate of about 6,900 viable seeds per plant from my experiment (Table 3.7).

With the amount of overlap between *R. conicus* and first generation *U. solstitialis*, the negligible level of co-occurrence is surprising: only 18 capitula were attacked by both, out of 967 capitula attacked by at least one of them in the experiment. Of these 18 capitula, only 16 actually exhibited larvae of both in the experiment (Table 3.6) At the survey, only a single capitulum exhibited both agents, out of 93 capitula attacked by either. It is possible that more capitula are attacked by both species, but that *U. solstitialis* larvae get predated upon by *R. conicus* before they manage to harden a gall (Harris, 1989a) leading to underestimates in my study. Alternatively, Woodburn & Sheppard (1995) claim *U. solstitialis* actively avoid ovipositing in capitula containing *R. conicus*. Although most of the time not all *C. nutans* capitula were attacked (neither at the experimental site nor at the survey site), it is possible that capitula which represent a preferred resource get occupied by *R. conicus*, while *U. solstitialis* either avoids these, or gets predated upon with no evidence left of its attack. Either way, potentially fewer *U. solstitialis* emerge from the generation which coincides with *R. conicus* than would be expected in the absence of *R. conicus* (see Section 4.1), which leaves later developing *C. nutans* capitula exposed to potentially less *U. solstitialis* attack than what would have been expected in the absence of *R. conicus*. Although a genuine low occurrence of *U. solstitialis* early in the season has yet to be ruled out as causing the low first generation, there is good indication that high densities of *R. conicus* early in the season reduce *U. solstitialis* numbers in the first generation in Australia (Woodburn, 1996a).

Interestingly, McNeill & Fletcher (2005) recorded 17% co-occurrence of *R. conicus* and *U. solstitialis* in a sample of 105 mainly primary *C. nutans* capitula from Canterbury; only about 2% of capitula had *U. solstitialis* alone, and 81% had *R. conicus* alone. Attack by *R. conicus* in their study occurred from late October, and by *U. solstitialis* from mid November, and sampling commenced at the end of October and ceased in mid December (however, non-ripe capitula were also collected). In comparison, in my study, the first *C.*

nutans capitulum ripened on 19 December. Over three seasons (2004-05 to 2006-07) in Canterbury, I have not encountered such early flowering *C. nutans* plants as reported by McNeill & Fletcher (2005); but the presence of *R. conicus* and *U. solstitialis* this early in the season is compatible with my observations.

Seed destruction per larva varied a lot between insect densities of four and densities of 5 or more individuals of less per capitulum (Figure 3.8). It is highly likely that this pattern is an artefact of the nature of linear regression, such as that used to estimate seed production, at the lower and higher ends of the spectrum. The majority of capitula were from the lower end of insect density in both insects, which may explain this pattern. Alternatively, this pattern may be the result of truncation; negative values of seed destruction were not allowed, and were replaced with zeroes. This artificially rises the mean, and more so at the lower insect densities, in which the vast majority of negative values arose. It is also possible that the pattern is real, i.e., that at low densities seed destruction per larva is indeed higher than at high densities. This could rise from intra-specific competition for limited resources between larvae at high densities. However, intra-specific competition is less likely to explain the pattern in the case presented here, because I present final densities, i.e., post any effect of such competitive interactions. Capitulum position and time in the season did not contribute to the variation in seed destruction (Figure 3.9; note that *R. conicus* attack late in the season is over represented by low densities, hence the higher seed destruction per larva). My sample accounts for the entire season, all capitula positions, and a high range of insect densities per capitulum. Therefore I trust my grand mean estimates of seed destruction per larva are representative and reliable. My estimate of seeds lost per *R. conicus* larva, 34.3, is similar to previous estimates from New Zealand of 29.6 (Popay *et al.*, 1984) and 27.7 (Kelly *et al.*, 1990). It is also similar to the 29 seeds per larva estimate from Australia (Woodburn, 1996a, p. 410). In Europe, loss to *R. conicus* was estimated to be 26 seeds per larva (Sheppard *et al.*, 1994). Seed loss per *U. solstitialis* larva was estimated to be 6.9 in Australia (Woodburn, 1996b), similar to the 7 estimated in Europe (Sheppard *et al.*, 1994), but both much lower than the 21.3 estimated in my experiment. In light of this, the total seed reduction of ~9% in the experiment and ~7% in the survey (Table 3.7) are highly disappointing. The 14% and 4% seed reduction by *R. conicus* in the experiment and the survey respectively, is similar to the previous estimate for Canterbury of 3% (Kelly *et al.*, 1990). In an Australian site with *R. conicus*, for example, *U. solstitialis* was estimated to reduce seed production by only 11% (Woodburn, 1997), much lower than the estimates for its effect when acting on its own (Woodburn & Briese, 1996). The resulting 23% total seed reduction in my experiment (and

only 11% at the survey site) is far short of the levels (~69%) estimated as required to bring *C. nutans* populations in New Zealand to decline (Shea & Kelly, 1998).

Thirty five years from initiation of the biocontrol programme, and three agents later, *C. nutans* is still a major weed in parts of New Zealand. The main finding of the study presented here is that *C. nutans* overall seed destruction hardly improved, at least in Canterbury, through the introduction of a potentially highly effective agent, *U. solstitialis*. This result highlights that multiple agents do not always benefit biocontrol.

Chapter 4: Interactions between nodding thistle biocontrol agents

4.1[1] Interactions between nodding thistle seed predators

4.1.1 Introduction

Nodding thistle, *Carduus nutans*, is a monocarpic weed of Eurasian origin, which relies solely on seeds for reproduction. In New Zealand, it has been shown that the high fecundity of the plant is an extremely important factor in its success (Shea *et al.*, 2005), and should therefore be the stage targeted for biological control. In light of this, the introduction to New Zealand of nodding thistle receptacle weevil, *Rhinocyllus conicus* (Froehlich) in 1972 (Jessep, 1989a), following its success in controlling nodding thistle in North America, might have been expected to reduce the weed's densities. The weevil larvae feed on the receptacle of developing buds and thus prevent seed formation. *Rhinocyllus conicus* is a highly fecund insect and distributes its many eggs on many capitula, and was therefore suggested as the most appropriate first choice of nodding thistle biocontrol agent to be introduced into an invaded range (Zwölfer, 1973). However, the North American success was not replicated in New Zealand, possibly due to the extended flowering season of nodding thistle in New Zealand compared to North America and to the weed's native range, which allows most inflorescences in New Zealand to escape weevil attack (Kelly & Wood, 1991).

With seed production remaining the target for biological control, a further seed predator was introduced in 1992, the woody-gall-forming tephritid fly *Urophora solstitialis* L. This fly has two generations a year, and thus extends the period in which nodding thistle is exposed to attack (Woodburn, 1996b). However, its first generation occurs simultaneously with *R. conicus* (normally) single generation and, despite a claim that *U. solstitialis* actively avoids ovipositing in *R. conicus*-attacked capitula (Woodburn & Sheppard, 1995), the two species are known to co-occur in the same capitula (Zwölfer, 1973; Harris, 1989a; Woodburn, 1996a; McNeill & Fletcher, 2005, R. Groenteman, pers. obs.). Conflicting hypotheses exist as to which of the two seed predators is likely to be a superior competitor and which is likely to produce better control: Zwölfer (1973) claimed *R. conicus* is the inferior competitor, and thus would not jeopardise a future introduction of *U. solstitialis* in case seed reduction was not satisfactory. The galls formed by *U. solstitialis*, on the other hand, render a considerable

[1] Section 4.1 has been published and may be cited as (Groenteman *et al.*, 2007). The version presented here has been slightly modified from the published version. Some aspects of this work which had to be omitted due to space limitation in the published version are expanded in Appendix A.

amount of the receptacle unsuitable for other herbivores and would therefore jeopardize future introductions of *R. conicus*. (Zwölfer, 1973). In contrast, Harris (1989a) claimed that woody gall formers should be the first choice for biological control of Cardueae weeds, because (1) the strong metabolic sink they produce utilises resources from the whole plant, not just the receptacle, (2) they are highly gregarious and (3) their limited dispersal assists in establishing populations while reducing dispersal-related mortality. Furthermore, Harris's advice that no insect should be introduced that might reduce the impact of woody gall formers, relates to a concern that receptacle feeders, such as *R. conicus*, were likely to displace gall formers, such as *U. solstitialis*. Harris further pointed out that Zwölfer's criteria should also have led to selection of *U. solstitialis* as the first agent to introduce, because this is the inferior competitor – it is displaced by *R. conicus* when they attack simultaneously, but not when *R. conicus* attack comes after the gall had hardened (Harris, 1991). Experimental evidence on interspecific competition between the two species is sparse generally (e.g. Moeller-Joop & Schroeder, 1986, in *Carduus acanthoides*), and non-existent for New Zealand. Retrospectively, derived from its impact in Australia, Shea & Kelly (2004) predicted that for a single biocontrol agent, *U. solstitialis* would have the strongest effect on nodding thistle population growth rate in New Zealand, but the lack of empirical data from New Zealand did not allow testing of this hypothesis.

This study quantifies the effect *R. conicus* has on *U. solstitialis* (and vice versa), and estimates how this might affect nodding thistle population growth rate. We hypothesised that the presence of *R. conicus* adversely affects the performance of *U. solstitialis*, and that the presence of *U. solstitialis* does not affect *R. conicus* performance.

4.1.2 Materials and methods

4.1.2.1 Plants and insects

Nodding thistle plants were grown in the University of Canterbury's glasshouses from seeds collected in summer 2005 in different parts of Canterbury. Seeds were sown in trays in a non-fertilised potting mix in late autumn 2005, and, in the winter were transferred to different sized pots, as well as transferred outdoors for vernalization. In early spring the plants were fertilised occasionally with nitrogen rich liquid fertiliser (Gro More® by Gro-Chem NZ Ltd, 15:2:12 N:P:K) to induce an annual life cycle (flowering in the first summer). When rosettes reached suitable sizes, they were planted out in the experimental plot, an uncultivated grassy area that was regularly mown, at the Landcare Research Lincoln campus. The thistles were caged in groups of nine and biocontrol agent treatments were assigned to them.

To obtain *U. solstitialis*, galled nodding thistle capitula were collected from South Canterbury in summer and autumn of 2005, and were kept refrigerated until 3 weeks before adults were required in the experiment. At this time they were put in emergence cages with a thistle plant. Peak emergence occurred around 3 weeks from exposure to spring temperatures and daylight. Emerging *U. solstitialis* were collected daily and kept refrigerated in a ventilated container, until required in the experiment.

Adult *R. conicus* were collected using a garden leaf sucker (a suction device designed to collect leaves, modified for insect collection by fitting a fine-mesh sleeve) during spring 2005 from South Canterbury and kept refrigerated in a ventilated container, until required in the experiment.

When needed at the experiment, mating *R. conicus* and *U. solstitialis* pairs were separated from the colony, to assure that reproductively active individuals are used.

4.1.2.2 Interactions between the two seed predators

In each plot, nine thistle rosettes of various sizes were planted in a 3 × 3 arrangement, 30 cm apart, in 45 plots (totalling 405 plants). Each plot was then caged in a 1.2 m × 1.2 m × 1.8 m (L:W:H) fine mesh cage. To estimate each rosette size, the longest leaf was measured at the day of planting, and then again upon bolting. When about 40% of the plants had bolted, each cage was assigned one of nine seed predator density treatments. The nine treatments were *R. conicus* at zero, low (one pair) and high (three pairs) densities and *U. solstitialis* at zero, low (five couples) and high (15 couples) densities, in all possible combinations (full factorial). The experiment was replicated five times in a randomized block design. Insects (in the same numbers) were again released in all the cages (except those assigned the zero *R. conicus* and zero *U. solstitialis* combination) around 3 weeks from the initial release, as a top-up, to replace mortalities. Plant height and number of primary, secondary and tertiary capitula were recorded fortnightly, and ripe capitula collected individually into paper bags, after having their individual height, position and external diameter recorded.

In the lab, the receptacle diameter was measured for each capitulum, and the seed predator larvae and adults were counted. The differences in insect counts (log transformed) between treatments were compared in a mixed model in R (R Development Core Team, 2006), with Poisson distribution (for a detailed explanation of model construction and output see Appendix A). All different plant and capitulum variables were included in the model as covariates, and non significant variables were excluded in backwards selection using the Chi-squared test to compare between models.

4.1.2.3 Simulation of the effect of the two seed predators on a nodding thistle population

Data from New Zealand on seed loss to biocontrol agents exist only for *R. conicus*, and were estimated at around 36% (Kelly & McCallum, 1990). Estimates for seed loss to *U. solstitialis* were taken from Australian datasets, showing this fly reduced seed rain by close to 70% (the value used in our simulation) several years after its introduction (Woodburn & Briese, 1996). These values were inserted into an existing matrix population model for nodding thistle in New Zealand (Shea & Kelly, 1998), and the thistle population growth rate (λ) was compared for the various combinations of biocontrol agents used in my study, assuming an additive effect.

4.1.3 Results

4.1.3.1 Interactions between seed predators

Of the 405 plants in the experiment, 208 bolted, and 202 produced capitula at 4.59 ± 0.19/plant (mean ±SE). Mean external diameter of the capitula (±SE) was 13.15 ± 0.14 mm. This is considerably smaller than nodding thistle plants typically found in New Zealand pasture. This could be due to the combination of poor soil at the experimental site, the caging of the plants (which may have reduced light intensity and rainfall and increased temperatures), and the timing of transplanting. Larger rosettes at planting were larger at bolting ($F_{1,205} = 197$, $r^2 = 0.49$, $P<0.0001$).

The mixed models show that both biocontrol agents preferred earlier, primary, higher, larger capitula on taller plants for oviposition (the difference between the best fit model and the next reduced model generating $\chi^2 = 5.517$, P=0.019, and $\chi^2 = 10.422$, P=0.001 for *U. solstitialis* and *R. conicus* respectively). The models' outputs are given in Appendix A.

Both at low and high density, *U. solstitialis* showed a trend (albeit marginally non-significant to non-significant) of a decrease in numbers in the presence of *R. conicus* (Table 4.1; Appendix A). In contrast, *R. conicus* increased in numbers in the presence of *U. solstitialis* (Table 4.1). Thus, in the absence of *U. solstitialis*, final *R. conicus* numbers corresponded with initial *R. conicus* density: at low initial density, final numbers were significantly lower than at high initial density. However, this difference disappeared when *U. solstitialis* was present, especially when it was present at the high density (see also Appendix A).

Table 4.1: Final density of *R. conicus* and *U. solstitialis* (individuals per 1000 capitula, fitted (backtransformed from log scale) means with 95% CI) in nodding thistle, given one of nine combinations of initial *R. conicus* and *U. solstitialis* density. The corresponding model outputs are given in Appendix A.

U. solstitialis initial density	*R. conicus* initial density		
	0	Low	High
	***R. conicus* final density**		
0	0	46.9 (17.9-123.4)	509.3 (258.5-1003.7)
Low	0	109.2 (46.2-258.1)	583.8 (272.4-1251.1)
High	0	362.0 (160.8-815.1)	425.5 (202.5-894.0)
	***U. solstitialis* final density**		
0	0	0	0
Low	0.63 (0.08-4.7)	0.06 (0.01-0.75)	0.28 (0.04-2.1)
High	4.9 (0.9-26.5)	3.8 (0.73-19.5)	2.5 (0.5-12.2)

4.1.3.2 Simulation of the effect of seed predators interaction on a nodding thistle population

Despite the top-up release of insects in the experiment, it appears that adults of both species suffered high mortality, especially *U. solstitialis*. The back-transformed means of insects per capitulum (Table 4.1) are very low compared to numbers occurring in natural populations. The high adult mortality and low insects per capitulum recovered are probably artefacts of the caging. Therefore, to estimate the effect of seed predator combinations on thistle population growth rate, the differences between combinations were used, rather than the actual means. Table 4.2 details the expected nodding thistle population growth rates at different combinations of *R. conicus* and *U. solstitialis*, as calculated by modifying Shea & Kelly's (1998) matrix. The greatest impact on thistle growth rates by a sole species of seed predator came from the highest density of *U. solstitialis* (in the absence of *R. conicus*). No combination of the two seed predators produced a population growth rate lower than this.

Table 4.2: Growth rate (λ)[1] predictions for nodding thistle populations exposed to various seed predator combinations, based on modifications to Shea & Kelly (1998) matrix model.

	R. conicus initial density		
U. solstitialis initial density	0	Low	High
0	2.2	2.2	1.7
Low	2.1	2.1	1.6
High	1.1	1.1	1.4

[1]λ values lower than 1 indicate a population in decline; equal to 1 is a stable population; and greater than 1 is a growing population.

4.1.4 Discussion

In the early stages of the debate on the choice between *R. conicus* and *U. solstitalis*, Zwölfer (1973) claimed the first choice of nodding thistle biocontrol agent to be introduced into an

invaded range should be *R. conicus*, partly because it is the inferior competitor, and would not jeopardise future introduction of *U. solstitialis*. However, apart from Zwölfer (1973), all evidence indicates that *R. conicus* is the superior competitor (Woodburn, 1996a; Woodburn & Briese, 1996; McNeill & Fletcher, 2005), or at least not inferior (Moeller-Joop & Schroeder, 1986). The present results support the superiority of *R. conicus* as a competitor, with *U. solstitialis* numbers per capitulum reduced by 22-90% in the presence of *R. conicus* (Table 4.1). Moreover, it appears from Table 4.1 that *R. conicus* benefited from the presence of *U. solstitialis*, as *R. conicus* numbers increased. Yet, from Table 4.2 it appears that nodding thistle biocontrol does not improve with this increase in *R. conicus* numbers.

The timing of attack has been identified by Harris (1989) as a major factor affecting the outcome of the inevitable competition between two species ovipositing at about the same time on the same niche. In New Zealand, *R. conicus* starts ovipositing slightly before *U. solstitialis* (McNeill & Fletcher 2005), and the present study shows that they prefer the same plant and capitula characteristics for oviposition. This exposes *U. solstitialis* larvae to predation by *R. conicus* before the gall hardens (Harris 1989). *Urophora solstitialis* larvae provide better nutrition compared to the receptacle tissue, thus such predation may be the reason for the increase in *R. conicus* numbers in the presence of *U. solstitialis* that was apparent (Table 4.1).

Other factors may affect these two biocontrol agent populations. In Australia, for example, *U. solstitialis* suffers from a new population regulation factor, where most adults emerge too early in the spring and die before any nodding thistle capitula are available for oviposition (Woodburn & Cullen, 1996). Sheppard & Woodburn (1996) stressed that, while in their native range *R. conicus* and *U. solstitialis* may be regulated to some extent by their natural enemies, in their introduced range, where they are released from such pressure, they may suffer from more intraspecific competition. Indeed, Woodburn (1996a) found that in early nodding thistle capitula that are very heavily attacked by *R. conicus*, no seeds are produced, but also no weevils complete their life cycle, as these capitula tend to abort prematurely.

Shea & Kelly (2004) predicted that of the three agents introduced to New Zealand (only two of these were considered in the present study), *R. conicus* would have the least effect on nodding thistle population growth. Not only does this weevil provide insufficient seed predation, but the present study now also implies that it out-competes the first generation of *U. solstitialis*, which inevitably results in a smaller second generation of the latter. Food supply (thistle capitula) is certainly not the limiting factor for this second generation of *U. solstitialis*.

It is apparent from Table 4.2 that *U. solstitialis* alone, at high densities, would be predicted to reduce nodding thistle population growth rate substantially (based on Australian impact data), while high densities of *R. conicus* cause a smaller reduction in thistle population growth rate due to interference with *U. soltitialis*. Yet, it is also obvious that no combination of *U. solstitialis* and *R. conicus* is predicted to cause populations in New Zealand to decline. Indeed, as suggested by Shea & Kelly (2004), it is the combination of *U. solstitialis* and a third biocontrol agent, a root crown feeder, which will be most likely to cause this thistle to decline.

4.2 Interactions between nodding thistle biocontrol agents

4.2.1 Introduction

The potential of herbivores to regulate plant populations and shape plant communities is nowadays widely acknowledged (Crawley, 1997). The majority of knowledge comes from studies on above-ground herbivory, but the effects of below-ground herbivory on plant population dynamics (McEvoy *et al.*, 1991; Müller-Schärer & Brown, 1995; Crawley, 1997) and on plant communities (Brown & Gange, 1989; Bardgett & Wardle, 2003; Blossey & Hunt-Joshi, 2003) are becoming increasingly recognized in recent years. Moreover, the importance of interactions between below- and above-ground herbivores are also beginning to gain recognition (Van Der Putten *et al.*, 2001; Bezemer *et al.*, 2003; Hunt-Joshi *et al.*, 2004).

For a long time root feeders were hardly used in weed biocontrol because they are logistically difficult to work with, and difficult to obtain in good numbers for releases (Blossey & Hunt-Joshi, 2003). Yet, root feeders have similar or better establishment rate than above-ground herbivores, and more root feeders appear to contribute to the suppression of the target weed than above-ground herbivores (Blossey & Hunt-Joshi, 2003). In addition, root feeders tend to be both more effective and more host specific than above-ground herbivores (Kok & Mays, 1991; Müller-Schärer , 1991; references within Blossey & Hunt-Joshi, 2003).

The literature is divided in predicting superiority in competitive interactions between below- and above-ground herbivores, and its implications for host plant suppression. Some theory and empirical evidence claim inferiority of below-ground herbivores and/or superiority of above-ground herbivores (Huffaker & Kennett, 1969; Gange & Brown, 1989; Moran & Whitham, 1990; Masters & Brown, 1992; Masters *et al.*, 1993; Masters, 1995a; Salt *et al.*, 1996; Masters *et al.*, 2001; Soler *et al.*, 2007), while others claim root herbivores are unaffected by above-ground herbivory (McEvoy *et al.*, 1991; McEvoy *et al.*, 1993; Strong *et al.*, 1995; Morón-Rios *et al.*, 1997; Hunt-Joshi *et al.*, 2004) or even superior to above-ground

herbivores (Bezemer *et al.*, 2003). The imbalance between studies claiming either way probably represents the type of systems normally selected for root herbivory studies – short term studies on annual plants and with external (not highly specific) root feeders; in such systems, root feeder superiority is less likely to be revealed (Blossey & Hunt-Joshi, 2003). Some claim root feeders may interfere with biocontrol when interacting with above-ground herbivores (e.g., Milbrath & Nechols, 2004b) while others claim an additive effect (Maron, 1998; Blossey & Hunt-Joshi, 2003; Gerber *et al.*, 2007b), a multiplicative (additive on a log scale) effect (Sheppard *et al.*, 2001), or (potential) sole responsibility for reaching (Shea *et al.*, 2005) or maintaining (McEvoy *et al.*, 1993) weed suppression.

It is clearly crucial for biological control systems to investigate which types of competitive interactions occur between multiple agents, to allow replicating successes, avoid replicating failures, and improve agent selection in future programmes.

The majority of the work on competitive interactions between below- and above-ground insect herbivores looked at leaf herbivores as the above-ground component (Huffaker & Kennett, 1969; Seastedt *et al.*, 1988; Gange & Brown, 1989; Moran & Whitham, 1990; Masters & Brown, 1992; Masters *et al.*, 1993; Masters, 1995a, b; Müller-Schärer & Brown, 1995; Bezemer *et al.*, 2003; Hunt-Joshi *et al.*, 2004); few studies involved reproductive tissue feeders above-ground (Smith & Kok, 1987; Müller-Schärer & Brown, 1995; Maron, 1998; Masters *et al.*, 2001; Milbrath & Nechols, 2004a). Reproductive parts differ from foliage as a resource in that they are considered a higher quality resource, are less abundant, and are fairly limited in the amount of time they are available, which increases the vulnerability of their specialized herbivores to changes in the resource (Janzen, 1971; Mattson, 1980; Straw, 1989; Crawley, 1997; Masters *et al.*, 2001). Reproductive-parts feeders are commonly used in weed biocontrol, thus it is surprising how little is done on these type of competitive interactions. In a situation such as the *C. nutans* biocontrol system, where two seed predators interact in one biocontrol system, it is of great importance to understand how the indirect interaction with the below-ground herbivore could affect the above-ground direct interaction. This could happen through mechanisms of phenological changes to the plant (which could improve or worsen synchrony with either seed predator) and/or changes to resource quality (which could affect different agents differently; not measured here).

Carduus nutans biocontrol system in New Zealand consists of two receptacle-feeders (*R. conicus* and *U. solstitialis*) and one root feeder (*Trichosirocalus horridus sensu* (Panzer); see Chapter 2 for clarification). The direct interactions between the two seed predators were discussed in Section 4.1. Only one study (Milbrath & Nechols, 2004a) has looked at the indirect interaction between *T. horridus* and one of the seed predators (*R. conicus*), but no

study has tested the interaction between *T. horridus* and *U. solstitialis*, nor the interactions between all three agents. The current study aimed to: (**a**) quantify the individual impact of *T. horridus* on *C. nutans* for the first time in New Zealand; (**b**) quantify the individual impact of *U. solstitialis* on *C. nutans* for the first time in New Zealand; (**c**) test for interactions between the three *C. nutans* biocontrol agents; and (**d**) quantify the effect of such interactions on *C. nutans* performance. I hypothesized that sub-lethal attack by the root feeder *T. horridus* would only change the amount of resource available to the seed predators, hence rendering the effect on *C. nutans* population additive. The interactions between the biocontrol agents were tested in a controlled caged experiment, and the effect on thistle populations was tested using a matrix model simulation.

4.2.2 Methods

4.2.2.1 Plants and insects

Carduus nutans rosettes that failed to bolt in the previous season (seed predator interactions experiment; Section 4.1) were used at the same experimental plot, an uncultivated grassy area at the Landcare Research Lincoln campus. Most rosettes (98) were positioned suitably for the experiment. The remainder (34) rosettes were transplanted in late June 2006 (winter) to suit the design. The thistles were arranged (and later caged) in plots of four.

Adult *T. horridus* from *C. nutans* were collected in autumn (their reproductive period) 2006 from North and South Canterbury, and were caged with *C. nutans* leaves as food and oviposition substrate. Leaves were replaced every 2-4 days and were dissected under a stereo microscope for *T. horridus* eggs. Eggs were placed in Petri dishes on moist filter paper layered over wet cotton wool, and stored in darkness at 4.5°C (to prevent embryonic development, Kok & McAvoy, 1983) for up to 6 weeks (following Milbrath & Nechols, 2004a). Two weeks before they were required in the experiment, the eggs were transferred to natural temperature and daylight conditions. Embryonic development was followed daily and batches of 15 eggs with dark head capsules (24-48 h pre-hatch) were then aspirated with water into Pasteur pipettes. The pipette tips were wrapped with Parafilm™ and they were stored back at 4.5°C for up to one week, after which they were used in the experiment. Hatched *T. horridus* larvae at different developmental stages, extracted from field-collected *C. nutans* rosettes (collected in winter 2006 from sites in North, Mid and South Canterbury) served as an additional source.

To obtain *U. solstitialis*, galled nodding thistle capitula were collected from South Canterbury in summer and autumn of 2006, and were kept refrigerated until 3 weeks before

adults were required in the experiment. At this time they were put in emergence cages with a thistle plant. Peak emergence occurred around 3 weeks from the move to spring temperatures and daylight. Emerging *U. solstitialis* adults were collected daily and kept refrigerated in a ventilated container, until required in the experiment.

Adult *R. conicus* were collected from *C. nutans* plants in North and South Canterbury during spring and summer 2006 using a leaf sucker, and were kept refrigerated in a ventilated container, until required in the experiment.

4.2.2.2 Interactions between the three biocontrol agents: field experiment

In each plot, four *C. nutans* rosettes of various sizes were planted in a 2 × 2 arrangement, 30 cm apart. There were 32 plots in total (128 plants). Two rosettes in each plot were randomly assigned '*T. horridus* present' treatment. Batches of 30 *T. horridus* eggs at 24-48 h pre-hatching (from two Pasteur pipettes) were placed on to the crowns of the assigned rosettes and covered with moist filter paper to prevent dehydration, and with a pot to protect them from frost. The eggs were monitored and filter paper moistened every 2 days. Longest leaf length in mm was measured at the time of egg application (24 June 2006). Nine days after egg placement, there were no signs of hatching, and the eggs were all assumed dead due to extreme cold weather (the coldest mean monthly temperatures in 34 years, NIWA, 2007), at which point the assigned rosettes were infested with batches of 20 *T. horridus* larvae, placed onto the crowns, and covered with moist filter paper. *Trichosirocalus horridus* infestation density was selected to produce moderate levels of infestation based on field observations (Chapter 3), and assuming around 2/3 hatching success; thus infested plants were expected to suffer damage, yet survive and reach flowering. Flowering plants were required in the later stage of the experiment, where indirect interactions between *T. horridus* and *C. nutans* seed predators were to be tested. Longest leaf length was measured again in the spring (3 November 2006, four months after *T. horridus* egg application). By late November 2006 (late spring) a large proportion of mortality occurred, mainly in plants infested with *T. horridus*, and the number of plots for the experiment was reduced to 20, in which it appeared there was a good chance (based on rosette size) for at least one infested plant and one un-infested plant to bolt. In three cases, an un-infested plant had to be transplanted into a plot to achieve this. As a result, 74 plants in 20 plots of 3 – 4 were followed from the end of November onwards, regardless of whether they bolted or not. Longest leaf length was measured again on 6 December 2006 (five months after *T. horridus* egg application), and once more, for individual plants, upon bolting. When about 40% of plants had bolted, each plot was caged in a 1.2 m × 1.2 m × 1.8 m (L:W:H) fine mesh cage and each cage was assigned one of four seed predator

treatments. The four treatments were: *R. conicus* present (one pair) or absent, combined factorially with *U. solstitialis* present (seven pairs) or absent. The experiment was replicated five times in a complete randomized design for the seed predators in the main plots (cages), and with *T. horridus* infestation as sub-plots (randomized within the main plots). Prior to seed predator release, the plants were sprayed with Permethrin (6.3g/kg active ingredient in the form of a vapour; Bug Bomb™ by KiwiCare®) to kill existing *R. conicus*. This is meant to be a very short-lived insecticide. Seed predators were first released three days after the Permethrin treatment, but failed to establish. Seed predators were released five more times at the same densities, at different intervals (depending on availability) within the following six weeks. *Urophora solstitialis* established, but *R. conicus* failed to. It is suspected that some ingredient in the formulation, other than the active ingredient, caused *R. conicus* death through feeding on foliage (*U. solstitialis* does not feed on foliage and was only affected for as long as the active ingredient was effective). Rigorous washing of all foliage was insufficient to improve *R. conicus* survival, and weevils eventually survived in only three cages assigned to two different treatments. *Rhinocyllus conicus* was therefore ignored in the analysis. Plant height, the number of stems and the number of primary, secondary and tertiary capitula were recorded fortnightly and stems were tagged and numbered. Ripe capitula were covered individually with fine mesh and later collected into paper bags, after having their individual height, stem tag number and position recorded.

In the lab, external diameter and receptacle diameter were measured for each capitulum and seed predator (mainly *U. solstitialis*) larvae and adults were counted. Actual seed counts were not taken, since pollinators were unable to gain entry to the cages.

Potential seed production was estimated per capitulum based on receptacle diameter, using the formula by Popay *et al.* (1984); seed destruction by *U. solstitialis* was estimated by multiplying actual *U. solstitialis* counts per individual capitulum by the estimate of 21.3 seeds destroyed per larva from Chapter 3. The resulting estimates were summed per plant. It needs to be acknowledged that seed destruction estimate in this manner is limited in that, both potential seed production and seed destruction per *U. solstitialis* larva, are derived originally from the same formula by Popay *et al.* (1984).

4.2.2.3 Interactions between the biocontrol agents: simulation of biocontrol agents effect on *C. nutans* population growth rate

An existing size –structured matrix population model for *C. nutans* in New Zealand (Shea & Kelly, 1998), which was recently modified by Jongejans *et al.* (2008), was manipulated to estimate the effect of *T. horridus* and *U. solstitialis* on *C. nutans* population growth rate (λ).

Manipulations to simulate *T. horridus* effects included incorporation of 15% reduced growth (averaged across all size categories, recorded in my study, averaged between four and five months from application of *T. horridus* eggs). Reduced growth changes the probability of plants moving from one size category to the next, and subsequently the probability of flowering. In addition, 9% increased mortality and 33% reduced seed production by surviving rosettes infested with *T. horridus* (recorded in my study) were incorporated into the model. Estimates for seed loss to *U. solstitialis* from my study included 28% in the absence of *T. horridus* and 17% in its presence; in addition an estimate from an Australian dataset was used, showing *U. solstitialis* reduced seed rain by close to 70% (Woodburn & Briese, 1996). These effects were incorporated into the model by reducing the reproduction elements in the matrix. Thistle population growth rates were compared between the various combinations of biocontrol agents. The updated base matrix (Jongejans *et al.*, 2008) is provided in Appendix A.

4.2.2.3 Statistical analysis

Statistical analysis was done using R (R Development Core Team, 2006).
Length of the longest leaf in mm at the time of *T. horridus* egg application and at four months later (3 November) were subjected to a 'Before-After, Control-Impact' (BACI) calculation (Green, 1979; Hurrell *et al.*, 2001), in which the impact of *T. horridus* was assessed by comparing the changes in longest leaf length of infested ('impact') and un-infested ('control') plants between the time of application ('before') and four months later ('after').
The value

$$\frac{(At/Bt)}{(Ac/Bc)}\times 100$$

was calculated for each of the 32 plots, where *B* and *A* are mean leaf lengths per plot at the time of *T. horridus* application (before) and four months later (after) respectively, and *t* and *c* denote treated and control subplots respectively. The BACI value defined in this way is the change in longest leaf length of infested *C. nutans* rosettes expressed as a percentage of the change in leaf length of un-infested rosettes. The BACI values were $\log_{10}$ transformed and analysed using a t-test. A BACI analysis was also performed on longest leaf length at five months after *T. horridus* egg application (6 December) in comparison to the length at the time of application. This was performed on 20 plots.

The effect of *T. horridus* on plant survival (logit transformed), on plant growth (leaf length at the time of egg application, log transformed), number of stems (log transformed), plant height and number of capitula per plant (log transformed), and on estimated seed

production per plant (log transformed), were tested in a series of mixed effect models using the 'lmer' command (lme4 package) in R. Similarly, the effect of *T. horridus* on the number (log transformed) of *U. solstitialis* individuals recovered per capitulum and the effect of *T. horridus* on the proportion (logit transformed) of seed destroyed by *U. solstitialis* per plant were tested in mixed effect models using the 'lmer' command. All relevant explanatory variables were included in the models as covariates; variables that were significantly affected by *T. horridus* were not included as covariates. Non-categorical covariates were standardized (i.e., divided by the variance after subtraction of the mean); this made the magnitudes of their effects comparable, despite being measured in different units. Non-significant variables were excluded from the models by backwards selection, using the Chi-squared test to compare between models. Only the best-fit minimum models are presented. Since different response variables were measured at different times in the season, the relevant covariates included in their initial models differ. In some cases, an observation had a missing value for a covariate that remained in the final model, or an observation did not qualify to be included in the model (e.g., plants in which all capitula were aborted did not qualify for seed production tests); as a result, tests differ in sample size. Plots and, where appropriate, plants and stems were included in the models as random grouping factors. Where binomial or Poisson error distributions were used, the 'lmer' command automatically corrects for overdispersion in the data if required. The 'lmer' command uses restricted maximum log-likelihood (REML) to fit models. The model outputs are presented as tables of parameter estimates, in which the intercept estimate includes the lowest level of each categorical variable (in an alphabetic or numeric order), and all following estimates appear as the **difference** from the intercept; the t-test or z-test for each parameter tests the null hypothesis that this **difference** is not statistically different from zero. Categorical variable were labelled such that the alphabetical or numerical value would offer a logical order of testing.

Where R did not generate p values for t-tests, these were calculated in Statistix (Version 8; Analytical Software, 2003).

4.2.3 Results

4.2.3.1 Effects of *T. horridus* on *C. nutans* growth, survival and damage

Four months after *T. horridus* application, infested rosette survival was 86% (back-transformed fitted mean), marginally non-significantly lower than survival of un-infested rosettes, (95%; Table 4.3); this apparent increased mortality was experienced despite the use of moderate *T. horridus* density, which was intended to induce only sub-lethal effects. Infested and un-infested plants did not differ significantly in longest leaf length at the time of

T. horridus egg application (Table 4.4; Figure 4.1 left bars); transplanted plants had significantly longer leaves (Table 4.4). BACI analysis showed that surviving infested rosettes at four months after *T. horridus* application were significantly smaller relative to un-infested plants (t_{29} = -2.608, P = 0.014; Figure 4.1, middle bars). Their longest leaf length was, on average 86% (back-transformed fitted mean) the length of un-infested plants. At five months from application, this difference was slightly increased and leaf length of infested plants was on average 83% the length of un-infested plants (t_{18} = -3.787, P = 0.001; Figure 4.1, right bars).

Table 4.3: Difference between *T. horridus* infested and un-infested *C. nutans* plants in rosette survival (logit transformed) at four months from *T. horridus* egg application: generalized linear mixed effects model with a binomial distribution and with plots as a grouping factor. Recorded on 3 November 2006 for 128 plants in 32 plots.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	2.982	0.570	5.233	<0.001
T. horridus	1	-1.177	0.615	-1.914	0.056
Longest leaf at egg application	1	0.932	0.441	2.114	0.035

[1]Intercept: no *T. horridus*

Table 4.4: Difference between *T. horridus* infested and un-infested *C. nutans* plants in longest leaf length (in mm; log transformed) at the time of *T. horridus* egg application: generalized linear mixed effects model with plots as a grouping factor.
Measured from 128 plants in 32 plots.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
Intercept[1]	1	4.784	0.043	110.83	<0.001
T. horridus	1	0.018	0.048	0.38	0.705
Transplanting	1	0.211	0.062	3.38	0.001

[1]Intercept: no *T. horridus*; not transplanted.

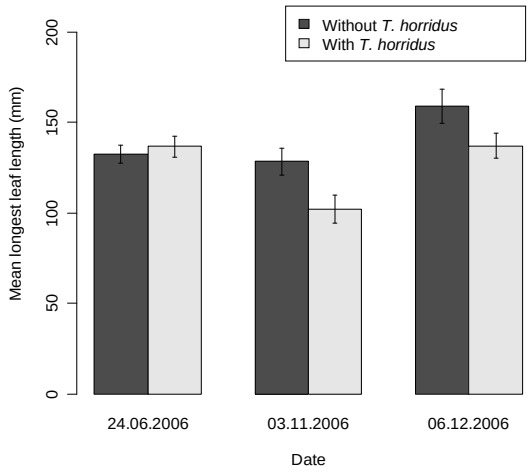

Figure 4.1: Raw mean (±SE) length in mm of *C. nutans* rosette longest leaf, measured on three occasions – at the time of *T. horridus* egg application (24 June 2006; 128 plants), at four months after application (3 November 2006; 128 plants) and at five months after application (6 December 2006; 73 plants). Light bars represent plants infested with *T. horridus*, and dark bars represent un-infested plants.

Among plants that survived and bolted successfully, plants infested with *T. horridus* tended to bolt, on average, more than six days earlier than un-infested plants, but this tendency was non-significant; larger plants (with longer longest leaf at the time of *T. horridus* egg application) bolted significantly earlier (Table 4.5). Infested plants produced on average 1.8 (back-transformed fitted mean) stems per plant, significantly more than the 1.06 stems per un-infested plant (Table 4.6).

Table 4.5: Difference between *T. horridus* infested and un-infested *C. nutans* plants in date of bolting (Julian days): generalized linear mixed effects model with plots as a grouping factor. Recorded from 60 plants that bolted successfully, in 20 plots.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
Intercept[1]	1	351.538	4.077	86.22	<0.001
T. horridus	1	-6.414	4.799	-1.34	0.189
Transplanting	1	-12.358	5.634	-2.19	0.035
Longest leaf at egg application	1	-9.940	2.643	-3.76	<0.001

[1]Intercept: no *T. horridus*; not transplanted.

Table 4.6: Difference between *T. horridus* infested and un-infested *C. nutans* plants in the number of main stems (log transformed): generalized linear mixed effects model with a Poisson distribution and with plots as a grouping factor.
Recorded from 62 plants that bolted successfully, in 20 plots.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	0.056	0.169	0.331	0.741
T. horridus	1	0.587	0.216	2.712	0.007

[1]Intercept: no *T. horridus*

At their peak height, infested plants were shorter on average by 126 mm than un-infested plants (Table 4.7). This difference was significant. Larger plants at the time of *T. horridus* egg application grew significantly taller, and plants that bolted at a later date grew significantly shorter (Table 4.7). Infested plants produced on average 5.0 capitula per plant, significantly less than the 6.6 on un-infested plants; larger plants at the time of *T. horridus* egg application produced significantly more capitula; transplanted plants and plants that bolted at a later date produced significantly fewer capitula (Table 4.8). Seed output per *T. horridus* infested plant was on average 33% lower than that of un-infested plants (Table 4.9). This difference was significant.

Table 4.7: Difference between *T. horridus* infested and un-infested *C. nutans* plants in peak plant height (in mm): generalized linear mixed effects model with plots as a grouping factor.
Measured from 58 plants that bolted successfully, in 20 plots.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
Intercept[1]	1	682.89	41.99	16.263	<0.001
T. horridus	1	-126.06	36.65	-3.439	0.001
Longest leaf at egg application	1	71.35	24.40	2.925	0.006
Bolting date	1	-130.13	24.33	-5.349	<0.001

[1]Intercept: no *T. horridus*

Table 4.8: difference between *T. horridus* infested and un-infested *C. nutans* plants in final sum (log transformed) of capitula produced: generalized linear mixed effects model with a Poisson distribution and with plots as a grouping factor.
Counted from 62 plants that bolted successfully, in 20 plots.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	1.889	0.138	13.642	<0.001
T. horridus	1	-0.276	0.123	-2.253	0.024
Transplanting	1	-0.739	0.186	-3.962	<0.001
Longest leaf at egg application	1	0.383	0.072	5.314	<0.001
Bolting date	1	-0.387	0.085	-4.551	<0.001

[1]Intercept: no *T. horridus*; not transplanted.

Table 4.9: Difference between *T. horridus* infested and un-infested *C. nutans* plants in estimated seed output per plant (log transformed; estimated using a formula by Popay *et al.* (1984)): generalized linear mixed effects model with plants and plots as grouping factors. Estimated for 60 plants that produced capitula that reached full bloom, in 20 plots.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
Intercept[1]	1	6.904	0.163	42.26	<0.001
T. horridus	1	-0.401	0.135	-2.96	0.006
Transplanting	1	-0.956	0.199	-4.81	<0.001
Longest leaf at egg application	1	0.459	0.090	5.12	<0.001
Bolting date	1	-0.495	0.095	-5.23	<0.001

[1]Intercept: no *T. horridus*; not transplanted.

4.2.3.2 Effects of *T. horridus* on *U. solstitialis*

Only 1.2 *U. solstitialis* individuals were retrieved per capitulum from plants infested with *T. horridus*, significantly fewer than the 3.2 from un-infested plants (Table 4.10). More *U. solstitialis* individuals were found in larger and higher capitula, and fewer in capitula at a secondary position, and on plants which bolted at a later date (Table 4.10).

Table 4.10: Difference between *T. horridus* infested and un-infested *C. nutans* plants in mean *U. solstitialis* individuals (all instars) per capitulum (log transformed): generalized linear mixed effects model with a Poisson distribution and with plants, stems and plots as grouping factors.
Counted from 179 capitula from 37 stems on 27 plants that produced capitula in the 10 plots that were assigned "*U. solstitialis* present" treatment.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	1.160	0.576	2.014	0.044
T. horridus	1	-0.986	0.496	-1.987	0.047
Receptacle diameter	1	0.832	0.032	26.083	<0.001
Capitulum height	1	0.315	0.043	7.376	<0.001
Secondary position	1	-0.744	0.091	-8.175	<0.001
Tertiary position	1	0.558	0.178	3.134	0.002
Bolting date	1	-0.824	0.302	-2.733	0.006

[1]Intercept: no *T. horridus*; at a primary position.

4.2.3.3 Effect of *T. horridus* and *U. solstitialis* on *C. nutans* seed production

In the absence of *T. horridus*, seed destruction per plant by *U. solstitialis* was estimated 27% (9, 58) (back transformed fitted mean and 95% CI; Table 4.11), which was significantly higher than in plants exposed to *U. solstitialis* in the presence of *T. horridus* (16% (5, 42); Table 4.11).

Table 4.11: Difference in the proportion (logit transformed) of estimated seed per *C. nutans* plant destroyed by *U. solstitialis* in the presence and absence of *T. horridus*: generalized linear mixed effects model with a binomial distribution and with plots as a grouping factor. Estimated from 29 plants that produced capitula in 10 plots with *U. solstitialis* present.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	-0.999	0.681	-1.470	0.142
Trichosirocalus sp.	1	-0.658	0.035	-18.680	<0.001
Transplanting	1	-0.791	0.087	-9.050	<0.001
Longest leaf at egg[2] application	1	0.312	0.022	14.460	<0.001
Bolting date	1	-1.049	0.030	-35.360	<0.001

[1]Intercept: no *T. horridus*; not transplanted.
[2]*T. horridus* eggs.

4.2.3.4 Simulation of the effect of the biocontrol agents on *C. nutans* population growth rate

Based on the parameters recorded in my study, neither *T. horridus* nor *U. solstitialis* nor the combination of both are projected to bring *C. nutans* population to stop growing, let alone to decline. In all three scenarios the matrix population model generated λ values that are greater than 1, which translates to a growing population. The combination of *T. horridus* and *U. solstitialis* generates a lower population growth rate than either agents alone (Table 4.12); at the level of seed reduction in this scenario, mortality of 46% is required to bring *C. nutans* population to start declining ($\lambda = 0.99$); however, at field levels of *T. horridus* attack (Chapter 3), both greater reduction of growth and greater rosette mortality due to *T. horridus* could be expected. A best scenario combination of 15% reduced growth, 9% mortality and 33% reduced seed production due to *T. horridus* (recorded in my study) plus high seed loss to *U. solstitialis*, as reported from Australia (70%; not including adverse effect of *T. horridus* on seed destruction by *U. solstitialis*) appears able to bring *C. nutans* population to slowly decline. Table 4.13 presents an array of proportional seed destruction values required at various levels of rosette mortality due to *T. horridus* attack.

Neither *Urophora* *Trichosirocalus* both

Table 4.12: *C. nutans* projected population growth rates (λ^1) as affected by *T. horridus* and/or *U. solstitialis*.

	Reduced vegetative growth by *T. horridus*	
Reduced seed output by *U. solstitialis*	**0%**	**15%**[2]
0%	2.68	1.85[3]
27%	2.17	1.65[4]

	70%	1.29	0.92[5]

[1]λ values lower than 1 indicate a population in decline; equal to 1 is a stable population; and greater than 1 is a growing population.
[2]Includes 9% mortality.
[3]Includes 33% reduction in seed production due to *Trichosirocalus*.
[4]Includes 33% reduction in seed production due to *Trichosirocalus* and further 17% seed destruction by *U. solstitialis* in the presence of *T. horridus*.
[5]Best case scenario; assuming *U. solstitialis* effect is not decreased by *T. horridus*.

Table 4.13: Proportion of seed destruction required to bring *C. nutans* population to decline at growing rosette mortality, assuming 15% reduced growth and 33% reduced seed production in plants surviving *T. horridus* attack, and assuming multiplicative effects of T. horridus and seed predator/s.

Percent mortality due to *T.horridus*	Proportion of seed destruction required
9	65
20	56
30	45
40	28
50	4

4.2.4 Discussion

4.2.4.1 Effects of root herbivory by *T. horridus* on *C. nutans*

The interest in root feeders as weed biocontrol agents has increased substantially in the last 30 years, although their use is still lagging behind above-ground herbivores (Blossey & Hunt-Joshi, 2003). While seed predators can only affect weeds' reproductive potential by affecting seed output (and can sometimes encourage compensatory seed production; Davis *et al.*, 2006), root feeders can affect weeds by reducing plant survival, by delaying flowering, and by reducing seed output (Müller-Schärer , 1991). They can also affect the vigour, architecture, and competitive ability of their host plant.

Effect of root herbivory on plant survival

Arguably, the most important effect of root herbivores is increasing plant mortality (Woodburn, 1997; Maron, 1998, 2001; Sheppard *et al.*, 2001; Briese *et al.*, 2002; Davis *et al.*, 2006; Gerber *et al.*, 2007a, b); (but see also Sieburth *et al.*, 1983; Cartwright & Kok, 1985; Sheppard *et al.*, 1995). In the current study, plants infested with the root herbivore *T. horridus* experienced increased mortality, despite the use of moderate larval densities which were only intended to induce sub-lethal effects. However, this increased mortality was marginally non-significant (Table 4.3). Most likely if higher densities of *T. horridus* per rosette had been

used, higher levels of mortality and smaller *P* values for the contrast with un-infested plants would have been found.

Root herbivore effects on plant survival are not necessarily through direct killing. Sometimes, the adverse effect of root herbivory on the competitive ability of their host plants is the main mechanism by which they regulate plant populations (Crawley, 1997). In this respect, the loss of growth exhibited by plants in the current study by exposure to root herbivory, needs to be considered in context: in the experimental cages, grass was regularly clipped; whereas the smaller weakened rosettes may well have been smothered by vigorous spring growth in well managed pasture.

Effect of root herbivory on plant architecture

At sub-lethal densities, root herbivores can change plant architecture in several ways:

- Most profoundly, by inducing growth of secondary stems by destroying the apical meristem and apical dominance (Cartwright & Kok, 1985; Jessep, 1989b; Tipping, 1992; Milbrath & Nechols, 2004a). This effect was recorded in my study as well (Table 4.6);
- By decreasing (Nötzold *et al.*, 1998; Sheppard *et al.*, 2001) or increasing (Woodburn, 1997) plant height. In my study, infested plants grew significantly shorter (Table 4.7), in contrast with Woodburn (1997) who also studied the effect of *T. horridus* on *C. nutans*;
- Root herbivores can reduce stem diameter (Goldson *et al.*, 1987; Müller-Schärer , 1991; Sheppard *et al.*, 2001; Gerber *et al.*, 2007b) (not measured in my study); and
- They can induce reduction of resource allocation to above-ground plant parts (Cartwright & Kok, 1985; Steinger & Müller-Schärer , 1992; Nötzold *et al.*, 1998; Briese *et al.*, 2002) or increase allocation to reproductive parts (Gange & Brown, 1989) (not measured in my study).

The latter difference in allocation responses to root herbivory is associated with plant life history: biennial and perennial species may compensate for root herbivory by allocating biomass to below-ground storage tissue and regrowth at the following year (see Cartwright & Kok, 1985; Steinger & Müller-Schärer , 1992; Nötzold *et al.*, 1998; Briese *et al.*, 2002), unlike annual plants, which have no opportunity to compensate in future growth, and may thus respond to root herbivory by allocating whatever resources they have left towards reproduction (see Gange & Brown, 1989). *Carduus nutans* is a monocarpic facultative biennial, which could delay flowering and compensate for root herbivory. At low density of the root herbivore *T. horridus*, compensatory regrowth of *C. nutans* can choke a large larva to

death (Lamp & McCarty, 1982a). Plant biomass was not measured in my study, but the loss of above-ground growth due to below-ground herbivory, indicated by longest leaf length, was maintained within the growing season (BACI analyses; Figure 4.1), and yet, 80% of the surviving infested plants bolted despite their reduced growth (not different from 89% of un-infested plants; data not shown). Thus, there was no indication of compensatory response as a strategy.

Effect of root herbivory on plant phenology

Root herbivory, and more specifically, *T. horridus* herbivory on *C. nutans*, was found to delay flowering by one week (Milbrath & Nechols, 2004a) and even two weeks (Woodburn, 1997). A delayed and shortened flowering period was also found in purple loosestrife, *Lythrum salicaria* L. in response to root herbivory (Nötzold *et al.*, 1998). In the current study, plants exposed to root herbivory tended to bolt almost a week before un-infested plants if any, but this tendency was non-significant (Table 4.5). However, this could be linked to the moderate *T. horridus* density used in the experiment as, in the past it has been shown (Milbrath & Nechols, 2004a) that delayed flowering occurs at high *T. horridus* densities but not at low densities.

Effect of root herbivory on plant reproductive output

Root herbivore effects on host plant reproductive outputs are usually expressed as reduced capitula and/or seed numbers (Cartwright & Kok, 1985; Kok, 1986; Müller-Schärer , 1991; Prins *et al.*, 1992; Tipping, 1992; Woodburn, 1997; Maron, 1998; Briese *et al.*, 2002), but could also be expressed as lower weight per seed (Prins *et al.*, 1992; Sheppard *et al.*, 2001). Plants exposed to root herbivory in my study produced significantly fewer capitula than did unexposed plants (Table 4.8), and were estimated to produce 33% less seed (Table 4.9).

4.2.4.2 Effect of *U. solstitialis* on *C. nutans* seed production

Unlike the high levels of seed predation in Australia (close to 70%, Woodburn & Briese, 1996), the individual effect of *U. solstitialis* in the current study only reached 27% (Table 4.11). However, these were not naturally occurring populations of *U. solstitialis* and, although flies were released in the cages fairly frequently, their survival between releases was not followed. Therefore, it is not unlikely that *U. solstitialis* exhibits a higher actual field capacity to reduce *C. nutans* seed production.

4.2.4.3 Interactions between the root herbivore *T. horridus*. and the seed predator *U. solstitialis*

Competitive interactions between below- and above-ground herbivores

Despite the spatial, and in many cases, also the temporal separation between below- and above-ground herbivores, they interact. If the below- and above-ground herbivores are separated in time, the below-ground herbivore is usually the first to utilize the plant. This can cause changes to the plant resource, by several mechanisms, which could affect above-ground herbivores either positively or negatively. One mechanism by which the resource quality is increased for the above-ground herbivore is water stress. Root herbivory could reduce plant water uptake and induce water stress (Goldson *et al.*, 1987). The reduced water level in the foliage results in increased leaf carbohydrates (Chapin, 1991) and nitrogen concentration (Brown & Gange, 1989; Newingham *et al.*, 2007, two different mechnisms) which enhances, at least temporarily, folivores; thus folivore growth, (Gange & Brown, 1989), fecundity (Gange & Brown, 1989; Masters & Brown, 1992) and density (Masters *et al.*, 1993) could be enhanced by root herbivory.

An opposite mechanism may be seen in biennials and perennials, which could draw nitrogen to the damaged below-ground tissues (Steinger & Müller-Schärer , 1992). This is likely to reduce the resource quality for above-ground herbivores. In addition, mobilisation of root reserves towards compensatory regrowth in response to root herbivory early in the growing season is expected to come at the expense of later reproductive development (Steinger & Müller-Schärer , 1992). Thus, seed predators may encounter reduced resource quantity. Milbrath & Nechols (2004a) found that potted *C. nutans* plants exposed to root herbivory by *T. horridus* represented a lower-quality resource for *R. conicus* as indicated by higher *R. conicus* larval mortality on infested plants (although this effect was significant only at low *R. conicus* densities). No mechanism is suggested that could explain this response, and it is suspected that these results may not necessarily be replicable under field conditions, because potted thistles respond to stress worse than field-grown thistles (Sieburth *et al.*, 1983). Water-stressed potted plants have been previously linked to reduced *R. conicus* size and increased mortality (Dowd & Kok, 1983). Reduced nitrogen fertilization was also linked to increased *R. conicus* mortality (Dowd & Kok, 1983).

In my study, *R. conicus* response to root herbivory could not be tested, due to the unexpectedly long-lasting activity of the insecticide used prior to insect release. *Urophora solstitialis* appeared to be adversely affected by root herbivory in my study, with significantly fewer flies per capitulum in plants infested with *T. horridus* (Table 4.10). Unlike *R. conicus*,

whose eggs are deposited externally and their hatching success can be determined to calculate mortality, *U. solstitialis* eggs are deposited inside the capitulum, and mortality cannot be determined to relate to root herbivory. Thus reduced resource quality due to herbivory by *T. horridus* could not be tested for *U. solstitialis*. Also, it needs to be stressed that *U. solstitialis* is a woody-gall former, and the gall is a strong metabolic sink; hence resource quality reduction should not be expected to affect *U. solstitialis* and *R. conicus* similarly. It would have been valuable to establish whether a change in resource quality indeed occurs in response to *T. horridus* feeding, which affects the two seed predators differently. Specifically, since it has been shown (Section 4.1) that in the absence of root herbivory, *R. conicus* potentially outcompetes *U. solstitialis*, it is of great importance to understand whether the addition of root herbivory improves the competitive ability of the potentially more effective *U. solstitialis*.

Another mechanism by which root herbivory can change the plant resource for above-ground herbivores is by altering plant phenology. Milbrath & Nechols (2004a) found that the one-week delay in *C. nutans* flowering due to root herbivory, combined with the fixed oviposition period of *R. conicus*, costs *R. conicus* a non-replaceable direct reproductive loss. Such a delay in initiation of flowering, especially in the South Island of New Zealand, where *C. nutans* and *R. conicus* are not well synchronized in the first place (Kelly & Wood, 1991), is likely to result in even worse synchrony between them, but might contribute to reduced encounters between *R. conicus* and *U. solstitialis* early in the flowering season, which could possibly improve performance of *U. solstitialis*. In my study, root herbivory was non-significantly linked to earlier *C. nutans* flowering (Table 4.5), which under field conditions would have just the opposite effect.

Effect of the interaction between *T. horridus* and *U. solstitalis* on *C. nutans* seed production: plant level – experimental results

Briese *et al.* (2002) estimated that owing to the temporal separation between *Trichosirocalus briesei* and a stem borer, their effect on the target weed should be additive. In contrast, Milbrath and Nechols (2004b) could not determine whether the combined effect of *T. horridus* and *R. conicus* was more or less than the sum of the individual impacts. My study quantified for the first time the combined effect of *T. horridus* and *U. solstitialis* on *C. nutans*, and the results imply that, while *T. horridus* has some negative effect on *U. solstitialis* (fewer *U. solstitialis* per capitulum) and its effect (fewer seed destroyed per plant; Tables 4.10 and 4.11 respectively), the combined effect of the two agents on *C. nutans*, 45% reduced seed

production, is greater than the individual effect of either (33% by *T. horridus* and 27% by *U. solstitialis*). This combined effect is less than multiplicative, and seems closer to additive.

Effect of the interaction between *T. horridus* and *U. solstitalis* on *C. nutans* seed production: population level – simulation results

In the absence of impact data from New Zealand for *T. horridus* and *U. solstitialis*, a previous analysis using some parameters from Australia (Shea & Kelly, 2004) estimated that the combination of these two agents is the one most likely to bring *C. nutans* populations in New Zealand to decline ($\lambda = 0.8$). This estimate was based on the New Zealand *C. nutans* population model and New Zealand data for *R. conicus* (Shea & Kelly, 1998), with Australian impact figures for *T. horridus* and *U. solstitialis*, relying on the similarity of *R. conicus* impact between the two countries. The updated population model (Jongejans *et al.*, 2008) and the preliminary impact data from New Zealand (albeit from a caged experiment rather than naturally occurring populations), still imply that this combination of agents is not yet sufficient to bring *C. nutans* populations here to decline ($\lambda = 1.65$; Table 4.12). However, under natural conditions, higher attack by *T. horridus* is expected on larger plants (Chapter 3), grass competition is expected to increase mortality of small stunned rosettes (Popay & Medd, 1990), and in contrast, the presence of *R. conicus* is likely to reduce *U. solstitialis* impact (Section 4.1). Hence, the population growth rates presented in Table 4.12 only present a preliminary indication of the population level effects of *T. horridus* and *U. solstitialis* in New Zealand.

4.2.4.4 Conclusions

The potential of root herbivores to affect many aspects of their host performance and population dynamics, together with their good establishment rates and relatively high host specificity, sees an increase in their use as weed biocontrol agents (Dunn & Rizza, 1976; Rizza *et al.*, 1988; Kok & Mays, 1991; McEvoy *et al.*, 1991; Woodburn, 1997; Sheppard *et al.*, 2001; Blossey & Hunt-Joshi, 2003; Newingham *et al.*, 2007; Soler *et al.*, 2007). While in some cases root herbivores would suffice for weed control, in many other cases, additional agents are likely to be required, in which cases, the sequence of agent introductions could prove vital for programme success. Population models are particularly informative in decision making on sequence of agent introductions, yet are still rarely used prior to introduction (Davis *et al.*, 2006), and only slightly more commonly used post-introduction. On the one hand, a general approach to the order of introduction claims that, if a root herbivore **and** above-ground herbivores are considered, then the introduction of the root herbivore first could

potentially reduce the quantity and quality of the above-ground herbivores, if their resource is thus reduced by root feeding (Briese, 2006). On the other hand, biocontrol programmes prove over and over that exceptions to the rules are common; hence, generalisations make useful baseline but each case is unique. Pre-release studies of weed population dynamics and of potential biocontrol agents for garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande), for example, indicated that (**a**) neither a root herbivore, nor seed predators alone are likely to control the weed over the entire North American invaded range; that (**b**) the release of seed predators prior to a root herbivore could cause a compensatory increase in seed production; and that (**c**) such increase is not predicted if the root herbivore was to be introduced by itself, or **prior to** a seed predator (Davis *et al.*, 2006). The important lesson from the study by Davis *et al.* (2006) is that while successful establishment is fundamental to achieving successful control, it does not guarantee it, and by working primarily to improve establishment rate we may jeopardize programme success.

A 'rule-of-thumb' (i.e., that *R. conicus* an inferior competitor that should be introduced first and would not jeopardise further agents should any would be required, Zwölfer, 1973) was clearly unsuccessful in determining the best sequence of *C. nutans* seed predator introductions. It has erroneously predicted the nature of their interaction and the subsequent effect on controlling their target weed (see Section 4.1).

Based on impact estimates from Australia, Shea *et al.* (2005) predicted that *U. solstitialis* was likely to be the most effective sole agent against *C. nutans* in New Zealand; whereas my results indicate that the root-herbivore *T. horridus* would have the greatest effect here, just like Shea *et al.* (2005) predicted for Australia. However, the current study may underestimate the sole effect of *U. solstitialis* in New Zealand, if to judge from its performance in Australia.

Further research to improve *C. nutans* control in New Zealand in an active adaptive framework should concentrate on methods to encourage the combination of *T. horridus* and *U. solstitialis*, and to discourage *R. conicus*. Such methods may include on-farm practices that could give *U. solstitialis* an advantage over *R. conicus*. For example, farmers in New Zealand sometimes collect capitula infested with *U. solstitialis* to spread on their property. Storage of such capitula in dry and warm conditions over spring could enhance earlier emergence of *U. solstitialis*, which may improve its competitive ability over *R. conicus*.

Chapter 5: Multi targeting for management of 'sleeper' weeds: the role of associational susceptibility to a shared herbivore

> It is inevitable that problems associated with invasive alien plants will increase; the transition of some species from the status of "emerging" to "major" invaders is one component of the escalation of the problem. (Mgidi et al., 2007, p.185)

5.1 Introduction

Plant invasions are strongly correlated to human activity, especially travel, colonisation and trade (Sullivan *et al.*, 2004; Sullivan *et al.*, 2005; Lambdon & Hulme, 2006). Typically, a time-lag of several decades (and sometimes hundreds of years; Rejmanek, 1999) elapses between an exotic plant naturalization and its becoming invasive (Sullivan *et al.*, 2004; Lambdon & Hulme, 2006); (but see chapter 2 in Cousens & Mortimer, 1995). Thus, many exotic plant species that may appear harmless, hold the potential of becoming invasive. Bearing in mind the developments in human movement across continents since 1950, we can realistically expect the magnitude of new invasions to dramatically increase in the near future. More efficient management strategies are constantly called for, and this will only increase. Perhaps one of the most important aspects of increasing management efficiency lies with targeting sleeper weeds. Sleeper weeds (also known as emerging or incipient weeds; Olckers, 2004), are defined as plants in the early stage of invasion – naturalized species that have not yet expanded their populations exponentially (Williams & West, 2000). A currently non-expanding species could become invasive if dispersed or translocated to a more favourable environment, where no factors inhibit its exponential growth (Mgidi *et al.*, 2007). With human aided dispersal through long distances in short times, such translocations are only a matter of time. There is no reason to assume that naturalized species that have become invasive elsewhere would forever remain harmless in a new range; in fact, invasiveness elsewhere is the best predictor we currently have for weediness in a new range (Reichard, 2001; Rejmanek, 2001). It is slowly becoming realized (Culliney, 2005; Moran *et al.*, 2005) and proven (Olckers, 2004), that by targeting sleeper weeds at the early stage of invasion, the prospects of success are greatly increased. In addition, it is far more cost-effective to control several non-weedy species, even when it is not known which of these would eventually become a problem, than to wait for a small proportion of them to become weedy and only control those (R. E. McFadyen, pers. comm.). Hence, targeting sleeper weeds is a significant approach that calls for considerable research attention (Williams & West, 2000; Macdonald, 2004; Olckers, 2004; Moran *et al.*, 2005; Mgidi *et al.*, 2007). Biological control is often the

most suitable approach for targeting weeds that have not yet become invasive, but are too widespread for eradication to be considered (Delfosse, 2005). One approach for targeting sleeper weeds that has been practiced in South Africa for many years is opportunism in two aspects: (1) exploitation of successes from other countries against the same weed or a closely related one; and (2) while on overseas explorations for biocontrol agents for weeds of high priority, opportunistic collections of natural enemies of weeds of secondary importance were, and still are, the usual practice (Olckers, 2004).

Another way of targeting sleeper weeds, which is suggested here, is 'multi targeting' – targeting a group of weeds with an oligophagous or even a generalist herbivorous biocontrol agent. Although the recent literature has seen many calls to avoid using generalists (e.g., Louda *et al.*, 2003b) and to only use agents with a very narrow host range (e.g., Strong, 1997; Strong & Pemberton, 2000), here I argue that **under certain circumstances** (i.e., weedy groups not represented in the native and in the economically important flora), the use of agents with a wide host range, at the genus, sub-tribe, or even tribe level, can be safe, and is more likely to succeed; undoubtedly, it would also shorten the host testing process and increase the benefit-cost ratio.

It is not uncommon that a potentially effective biocontrol agent is rejected because its host range is not sufficiently narrow (some examples are: Syrett *et al.*, 1995; Fowler *et al.*, 2000; McFadyen & Weggler-Beaton, 2000; Moran *et al.*, 2005). Instead, a more specific agent that is less effective may be approved for release, inevitably resulting in only partial or even negligible pest control (Harris, 1991). Usually, further introductions of alien biocontrol agents are required in these cases to improve control. In a multi targeting approach, a main weed is targeted, for which the most efficient agent may be considered for release, even if its host range includes other closely related species. The target weed is more likely to be successfully controlled this way, and any suppression of other closely related species, especially sleeper weeds, is in fact welcome.

Groups that are taxonomically close to the pest are the most prone to non-target effects (Louda *et al.*, 2003b); more specifically, in weed biocontrol, non-target plants are more prone to non-target effects if they belong to the same genus or tribe as the target weed (Pemberton, 2000; Strong & Pemberton, 2000; Louda *et al.*, 2003b). Islands are, in many cases, characterised by a unique flora in which certain plant groups may not be represented. Thus, if a genus or a tribe of introduced weeds is not represented in the native flora, multi-targeting should be genuinely considered. Conflicts with beneficial crops should be taken into account, and possible future utilization of plants in the group should be explored before a generalist agent is considered. In New Zealand, several weeds have no close relatives in the native flora.

Examples are *Tradescantia fluminensis* Vell. (wandering Jew or wandering Willie, Order Commelinales), with no natives in the order (Standish, 2001); *Ageratina riparia* (Regel) (mist flower), with no native representatives in the tribe (Eupatorieae) and in the two most closely related tribes (Heliantheae and Helenieae; Barratt & Moeed, 2005); and thistles (discussed in detail hereafter).

To study the feasibility and possible application of the multi targeting approach in New Zealand, I used thistles, from the tribe Cardueae in the Asteraceae. There are at least 115 species in the tribe Cardueae in New Zealand, all of which are exotic, and at least 9 are economic weeds; 38 species in the tribe Cardueae that are not currently weeds in New Zealand, are recorded as weeds elsewhere in the world (Hawaiian Ecosystems at Risk Project & AgWest, 2007), and are hence considered potential sleeper weeds; 23 of them are already naturalised here (S.V. Fowler, pers. comm.). Two species that have not naturalised anywhere but in New Zealand and are not yet weedy here could be added to the list of potential sleeper weeds. There are no natives in the tribe Cardueae in New Zealand, and the only valued species are the minor crops globe artichoke, *Cynara scolymus* L., safflower, *Carthamus tinctorius* L., variegated thistle, *Silybum marianum* (L.) Gaertner, which is currently a weed but could potentially become a future crop (Bourdôt *et al.*, 2007), and some globe thistles, *Echinops* spp., which are used as ornamentals. A specificity analysis of insect herbivores attacking six weedy thistle species (from the genera *Carduus* and *Cirsium*) in Europe alone revealed six species-specific, four genus-specific, 46 sub-tribe-specific (sub-tribe Carduinae), and 16 tribe-specific (tribe Cardueae) herbivore species (Gassman, 2005). Thus, acceptance of herbivore species which show host specificity to the sub-tribe and/or tribe level greatly increases the potential for effective biocontrol agents to be found. Several biocontrol agents have already been introduced to New Zealand under three programmes, targeting three thistle species (*Carduus nutans* , *Cirsium arvense* , and *Cirsium vulgare*), none of which is currently under satisfactory level of control (i.e., all three thistles still require substantial application of other control methods). Thus, considering the number of current and sleeper thistle weeds, multi targeting emerges as a suitable approach for future biocontrol of this group in New Zealand.

Carduus nutans was the first thistle for which a biocontrol programme was initiated in New Zealand. Three biocontrol agents against *C. nutans* have been introduced to New Zealand between the early 1970s and early 1990s, all of which are not restricted to, but show clear preference for, *C. nutans* (Zwölfer & Harris, 1984; Moeller-Joop & Schroeder, 1986; Jessep, 1989b, a; Gassman, 2005). In a multi targeting approach, as in any other biocontrol

approach, severe damage to the target weed is the ultimate goal; multi targeting is distinct from other approaches by the extended goal of increased damage to non-target sleeper weeds.

In multi-targeting the target weed and the sleeper/s share a herbivore. Sharing a herbivore between two or more plant hosts could result in different scenarios, such as 'associational resistance', in which the association between palatable and unpalatable species could reduce herbivory on the palatable species (Wahl & Hay, 1995) or 'associational susceptibility', in which herbivory on a secondary host is increased in the presence of the primary host (Brown & Ewel, 1987). For multi-targeting it is essential that the presence of the target weed inflicts associational susceptibility of the sleeper/s to the shared herbivore, such that where the sleeper/s grow in association with he target weed they suffer no less, and preferably greater attack and damage by the shared herbivore, compared to where they grow in the absence of the target weed.

Associational susceptibility and its potential effect on plant population dynamics have been demonstrated in several studies (Futuyma & Wasserman, 1980; Parker & Root, 1981; Thomas, 1986; Brown & Ewel, 1987; Hjalten *et al.*, 1993; Wahl & Hay, 1995; Rand, 1999; White & Whitham, 2000; Rand, 2003), including in an *R. conicus* – thistle system (Rand & Louda, 2004; Russell *et al.*, 2007).

In this study, I hypothesised that the presence of the preferred target species (*C. nutans*) in a patch would result in heavier biocontrol agents attack in that patch, and consequently heavier damage to non-target thistle species in the same patch that are acceptable hosts. I selected three non-target thistle species, closely related to *C. nutans*: the two congenerics *Carduus pycnocephalus* and *Carduus tenuiflorus*, and the Carduinae subtribe member *Cirsium vulgare*. *Carduus nutans* biocontrol agents attack and damage inflicted on these non-target species, growing in the absence of vs. in close proximity to *C. nutans,* were followed in a controlled field experiment and in a field survey.

5.2 Methods

5.2.1 Study system

Carduus nutans represented the target species in the system. It is a monocarpic facultative-biennial, which can take an annual life history (Popay & Medd, 1990). Its rapid spread around New Zealand in the early 1950s, after a lag phase of several decades from its introduction (Delahunty, 1960), and its invasive and weedy potential made it a candidate for a biocontrol programme. Two of the three biocontrol agents introduced in this programme are receptacle feeders which target the reproductive potential of the weed by limiting its seed production.

These are the receptacle weevil, *Rhinocillus conicus* (Coleoptera: Curculionidae), introduced in 1972 (Jessep, 1989a) and the gall-forming fly *Urophora solstitalis* (Diptera: Tephritidae), introduced in 1990 (Harman *et al.*, 1996). Both biocontrol agents are not restricted solely to *C. nutans*, and *R. conicus* in particular is known to attack a large number of hosts within the tribe Cardueae (some examples include Zwölfer, 1965; Goeden & Ricker, 1977; Rees, 1977; Goeden & Ricker, 1978; Zwölfer & Harris, 1984; Turner *et al.*, 1987; Pemberton, 2000).

Carduus pycnocephalus, *C. tenuiflorus* and *C. vulgare* represent the non-target species in the system. *Carduus pycnocephalus* and *C. tenuiflorus* are both annual species, which start flowering about 4 weeks before *C. nutans* does, a time when *R. conicus* reproductive activity has already begun (Jessep, 1989a; pers. obs.). *Cirsium vulgare* shares the same life history characteristics as *C. nutans*, and starts flowering at about the same time or just days after *C. nutans*. *Rhinocyllus conicus* attack on *C. pycnocephalus* and *C. tenuiflorus* has been recorded in New Zealand (Popay *et al.*, 1984; Kelly, 1988; Jessep, 1989a; Kelly *et al.*, 1990; pers. obs.). *Cirsium vulgare* is not a favourable host for *R. conicus* development in New Zealand; in various field surveys I have only come across a single adult and four pupae of *R. conicus* on *C. vulgare*, and another five adults were found from *C. vulgare* capitula in the experiment described here – only two of which successfully completed their life cycle, and the remaining three did not emerge successfully and died within the capitulum. Jessep (1989a) too, did not list *C. vulgare* as a species supporting complete *R. conicus* development in New Zealand; nevertheless in the survey presented in this chapter I have encountered levels of *R. conicus* oviposition on *C. vulgare* which appear higher than could be associated with occasional migrating weevils; hence I estimate that *R. conicus* populations are sustained even in areas consisting of mainly *C. vulgare* and no *C. nutans*. Gall forming tephritids, such as *Urophora* spp., are considered highly host specific; most are considered oligophagous, but many are known to be monophagous (Freidberg, 1984; Harris, 1989b; Turner, 1996; Headrick & Goeden, 1998). *Urophora solstitialis* attack on *C. pycnocephalus* and *C. tenuiflorus* has not been reported from New Zealand, and I was only able to find one record of possible attack on *C. pycnocephalus* elsewhere (Goeden, 1974); in three years of field observations and capitula collections I have only come across *Urophora* sp. inside *C. tenuiflorus* capitula on a single occasion and at very low density (5%). *Cirsium vulgare* was not expected to be attacked by *U. solstitialis*; in fact, a *C. vulgare* specialist, *Urophora stylata* Fabricius, was introduced to New Zealand in 1999, (Fowler *et al.*, 2000). *Urophora stylata* (but no *U. solstitialis*) has been recorded from *C. pycnocephalus* in the native range in Europe (Sheppard *et al.*, 1991).

5.2.2 Plants and insects

The non-target species, *C. pycnocephalus*, *C. tenuiflorus*, and *C. vulgare*, and the target species *C. nutans* plants were grown in the University of Canterbury glasshouses from seeds collected in summer 2005 around Canterbury. In late autumn 2005 they were transplanted as small rosettes to the experimental plot, an uncultivated grassy area that was regularly mown, at the Landcare Research Lincoln campus.

To obtain *U. solstitialis* and *R. conicus*, attacked *C. nutans* capitula were collected from South Canterbury in summer and autumn of 2005, and were kept refrigerated until several days before adults were required in the experiment. At this time they were put in emergence cages with a *C. nutans* plant. It was found that *U. solstitialis* peak emergence occurred around 3 weeks from exposure to spring temperatures and daylight; this was considered too late for release in the experiment, as *C. pycnocephalus* and *C. tenuiflorus* were already abundantly flowering. Hardly any *R. conicus* emerged from the refrigerated capitula, yet *R. conicus* adults populated the experimental plot naturally. Thus, no intentional releases of biocontrol agents were carried out, and the density of biocontrol agents was not controlled for.

5.2.3 Biocontrol agent attack on non-target species in the presence and absence of *C. nutans*: a field experiment

At the experimental site, non-target species rosettes were organized into plots, each containing five *C. tenuiflorus* plants, five *C. pycnocephalus* plants and five *C. vulgare* plants in a 3 × 5 randomised arrangement, with 0.2 m between plants and 3 m between plots, for a total of 24 plots. Plots were grouped in blocks of four, with at least 5 m between blocks. Two plots in each block were randomly assigned a "*C. nutans* present" treatment, and in these, 10 *C. nutans* rosettes were planted 0.2 m apart, in two rows, 0.4 m away from the non-target plants. Field surveys (e.g., Chapter 7), have shown that specimens of different thistle species are commonly found growing in such distances in naturally occurring multi-species assemblies. Plant arrangement is illustrated in Figure 5.1. Altogether, the experiment included 480 plants (120 plants per species). The experiment was designed with six blocks (replicates), and four treatments per block (*C. nutans* present/absent, and cage present/absent to manipulate biocontrol agents density), but the cage treatment was cancelled due to difficulty obtaining sufficient biocontrol agents early enough in the season, when *C. pycnocephalus* and *C. tenuiflorus* were already bolting. Hence all plots remained uncaged.

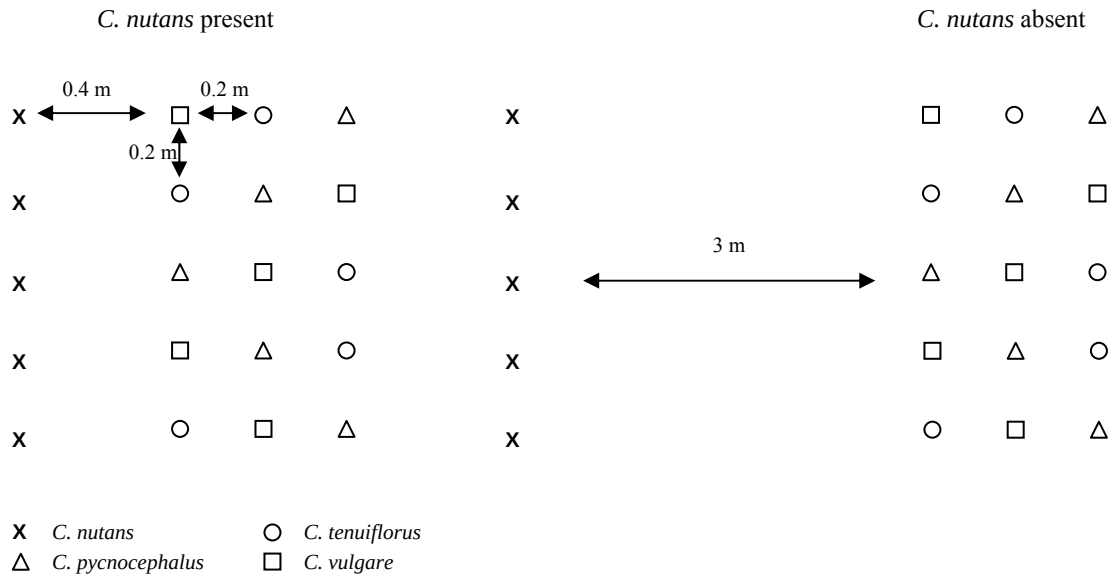

Figure 5.1: An illustration of two experimental plots with *C. nutans* either present (left) or absent (right).

The longest leaf of each rosette was measured at the time of bolting, and fortnightly thereafter I recorded plant height, total number of flower clusters (in *C. pycnocephalus* and *C. tenuiflorus*), their position, and total number of capitula. These data were used as covariates at the plant level; plant height and capitula sum were also used as plot level covariates (average plot height and plot sum of capitula combined for *C. pycnocephalus* and *C. tenuiflorus*). All ripe capitula were collected fortnightly and placed individually into paper bags. Capitulum height, the number of capitula in the cluster (for *C. pycnocephalus* and *C. tenuiflorus*; on the plant for *C. vulgare*), cluster/capitulum position, and capitulum external diameter were recorded for these individually collected capitula. Initially this was done for all ripe capitula on all plants; but as *C. pycnocephalus* and *C. tenuiflorus* growth exceeded the capacity to collect these detailed data, one *C. pycnocephalus* plant and one *C. tenuiflorus* plant were randomly selected in each plot, for which this detailed data collection continued. For the remaining plants, all ripe capitula were collected into a single bag per plant each fortnight, with no detailed information at the capitulum level recorded in the field; these are hereafter referred to as bulk collections. In one plot, where the amount of time required to individually collect capitula from a single plant equalled the amount of time it took to collect in this manner from 20 other plots jointly, the detailed collection was abandoned altogether, and only bulk collections were made. Very few *C. vulgare* plants flowered in the experimental plots (28 in total), and all their ripe capitula were collected individually. All individually collected capitula and sub-samples from the bulk collected capitula (up to 40 capitula per plant per census, if available) were later processed in the lab, where their receptacle diameter was

measured and the presence and number of any signs of attack by *R. conicus* were recorded (no *U. solstitialis* attack signs were found). *Rhinocyllus conicus* attack signs included egg cases, larval penetration holes, presence of any larval stages and adults, and typical feeding damage. Penetration holes, burrowed by 1st instar larvae, are sometimes the only evidence left of an oviposition event if the egg case has fallen off and no further larval development occurred within the capitulum. In *C. vulgare*, eggs and penetration holes were not counted; only their occurrence was noted as either nought, low, medium or high. The level of *R. conicus*-related feeding damage in attacked capitula was ranked from 0 to 3 (0 – none, 1 – little, 2 – medium and 3 – high damage), and any viable seeds were counted. In many cases capitula ripened faster than collections could keep up with, and by the time they were collected some seed would have already dispersed or been predated by goldfinches; in these cases seeds were not counted.

5.2.4 Biocontrol agent attack on non-target species in the presence and absence of *C. nutans*: a field survey

The pattern of *C. nutans* biocontrol agent attack on the same non-target species as in the experiment above was surveyed on a geographic spatial scale in the Canterbury district. Sites were located that hosted natural populations of the non-targets growing in the absence of *C. nutans*, and growing in close proximity to *C. nutans*. Although *C. pycnocephalus* and *C. tenuiflorus* could, in the past, "often be found growing in association with *C. nutans*" (Jessep, 1989a, p.340), many searches only yielded one region where this association could still be found, in North Canterbury (Culverden area, Kaiwara Rd., 42°49.111' S 173°02.983' E). *Cirsium vulgare* was also growing in this region. Thus, Culverden was one site, hosting all four species (the three non-targets and the target). Two sites were located on Banks Peninsula, Central Canterbury, one hosting *C. pycnocephalus* and *C. tenuiflorus*, (Christchurch Port Hills, Mt. Vernon Park, 43°35.713' S 172°34.885' E) and one hosting *C. vulgare* (Akaroa area, 43°47.724' S 172°58.876' E). *Carduus nutans* has been reported from Banks Peninsula (H.D. Wilson, W.R. Sykes, pers. comms.), but rarely, and I could not find it there; therefore Banks Peninsula was considered an area in which *C. nutans* is absent. Two additional sites were located in another part of Central Canterbury, near Rakaia Gorge, which hosted *C. vulgare* alone (Hoskyns Rd. 43°31.660' S 172°17.662' E), and *C. vulgare* adjacent to *C. nutans* (Coalgate area, 43°28.713' S 171°59.997' E). All sites were visited once a year (in 2005-06 and 2006-07) at the time of peak flowering of the relevant species; thus, Culverden site was visited twice a year – once at the peak of *C. pycnocephalus* and *C. tenuiflorus* flowering, and once at the peak of *C. nutans* and *C. vulgare* flowering. At each visit, about

100 ripe capitula were collected from the species at peak flowering; plants were randomly selected along an arbitrary walking transect and one capitulum at a primary position was collected per plant. In 2005-06, the site hosting *C. vulgare* and *C. nutans* at Rakaia Gorge did not have enough plants, and thus more than one capitulum per plant were collected, and practically all plants at the site were sampled. Ninety one *C. vulgare* capitula were collected on this occasion, and only 52 *C. nutans* capitula. All capitula were processed in the lab, where capitulum and receptacle diameters were measured and the presence and number of any signs of biocontrol agent attack were recorded. Biocontrol agent signs included *R. conicus* egg cases, larval penetration holes, larvae, adults and typical feeding damage, and *U. solstitialis* galls, larvae, and pupae. Plant density was not monitored in these surveys.

5.2.4 Statistical analysis

The effect of *C. nutans* presence and of capitulum, plant and plot level measurements on the probability of the non-target species to get attacked by *R. conicus* (logit transformed) as well as on the number of attack signs per capitulum (log transformed) were tested in a series of linear mixed effect models using the 'lmer' command (lme4 package) in R (R Development Core Team, 2006); all possible explanatory variables (but no interactions) were included in the models as covariates. The initial models included 12 fixed effects (only eight for *C. vulgare*) as explanatory variables. These were the treatment effect (i.e., *C. nutans* presence/absence); capitulum level covariates – external diameter (mm), height (mm) and position (in *C. vulgare*); cluster level covariates (in *C. pycnocephalus* and *C. tenuiflorus* only) – cluster position and sum of capitula in the cluster; plant level covariates – bolting date, longest leaf length (mm) at the time of bolting, plant height (mm) at the time of sampling, sum of clusters on the plant and sum of capitula on the plant; plot level covariates (for *C. pycnocephalus* and *C. tenuiflorus* only) – sum of capitula in the plot and average plot height (mm). Non-categorical covariates were standardized (i.e., divided by the variance after subtraction of the mean); this made the magnitudes of their effects comparable, despite being measured in different units. In addition, plants (nested within plots), plots (nested within blocks) and blocks were included as random grouping effects. Non-significant variables were excluded from the models in backwards selection using the Chi-squared test to compare between models. In some cases, a covariate was retained in the model despite being non-significant, if excluding it decreased the model fit significantly. All random effects were retained, even if they did not explain a significant amount of the deviance, because they comprise the structure of the experiment. Two *C. tenuiflorus* plants had a missing record for bolting date, and several capitula had missing observations for various covariates; as a result,

the sample size slightly differs between analyses, depending on the covariates that were retained in each model. Where binomial or Poisson error distributions were used, the 'lmer' command automatically corrects for overdispersion in the data if required. The 'lmer' command uses restricted maximum log-likelihood (REML) to fit models. The models' outputs are tables of parameters' estimates, in which the intercept estimate includes the lowest level of each categorical variable (in an alphabetic or numeric order), and all following estimates appear as the **difference** from the intercept; the t-test or z-test for each parameter is for the null hypothesis that this **difference** is not statistically different from zero. Categorical variables were labelled such that the alphabetical or numerical value would offer a logical order of testing.

Damage rankings were tested in proportional odds models, also known as cumulative logit models (Duncan & Young, 2000). In the proportional odds model, a regression model is fitted for the response variable (ranked categorical damage level), such that the cumulative probability of a response is increased damage ranking as a function of the predictor variables. The response variable, damage ranking, has four levels (none, little, medium and high), and these are separated by three cut points: the first separating capitula exhibiting no damage from the rest, the second separating capitula exhibiting no or little damage from the rest, and the third separating capitula exhibiting no, little or medium damage from the rest (i.e. capitula exhibiting high damage ranking). The model that is then fitted on the logit of these cumulative probabilities results in a series of J -1 regression lines (J being the number of rank categories) on a logit scale. Each line is described by one cut point intercept term and a vector of slope estimates (β, one slope estimate for each explanatory variable). In the case of damage ranking, the interpretation of the model is that the probability for a capitulum to be damaged at a certain level or lower is proportional to the change in a predictor variable X in the relationship: $e^{\beta(x_1-x_2)}$; the model assumes the different categories are affected similarly by the explanatory variables (Agresti, 1996). The strength of the proportional odds model is in taking into account the ordering of categories while making no assumptions about the intervals between them. (Duncan & Young, 2000). Here, I used the 'bayespolr' command (arm package) in R to fit the proportional odds models. The models included capitulum, plant and plot level explanatory variables as in the mixed effects models and, in addition, included the number of attack signs per capitulum; but they could not include the random grouping effects. Non-significant variables were excluded from the models in a backward selection (with the 'drop1' command in R) using the Chi-squared test to estimate variables significance.

In many cases it was observed that attack on the non-targets did not lead to any feeding damage inside the capitulum. The percentages of capitula exhibiting damage of those

that got attacked, in the presence and absence of *C. nutans* were tested in a series of Chi-squared tests. For *C. pycnocephalus* and *C. tenuiflorus*, the percentage of damaged capitula out of those attacked was also compared between early and late in the season (i.e., before and after *C. nutans* became available for *R. conicus* attack; 13 December 2005).

Capitula that had no feeding damage and no seed lost to goldfinch predation or wind dispersal were used to estimate relationships between capitulum external diameter and seed production. A linear regression was calculated for each species, following mixed effects analyses models using the 'lmer' command (lme4 package) in R with plants as a grouping factor. These regressions were used to estimate the seed output for each capitulum from its diameter; these were then summed to give the estimated seed output per plant. This estimate was done only for plants that had all their capitula collected individually and hence had all their capitula processed: 23 plants for each of *C. pycnocephalus* and *C. tenuiflorus*. In addition, the number of seeds lost at each damage category was estimated from attacked capitula that had no goldfinch predation marks and no wind dispersal signs. The mean proportion lost at each damage category was used to calculate seed loss estimates per plant for the same 23 plants per species. The proportion of loss at zero damage was calculated separately for un-attacked capitula and for attacked but un-damaged capitula. The proportion (logit transformed) of seed lost per plant were compared in mixed effects models using the 'lmer' command (lme4 package) in R with plant level and plot level explanatory variables included as covariates, and with block as a random grouping factor (plots were not included because only one plant was analysed per plot). Non-significant variables were excluded from the models in a backwards selection using the Chi-squared test to compare between models. Seed loss analyses were not done for *C. vulgare*, because not enough damaged capitua were available to produce a regression equation for the 'damaged' status.

The 'lmer' model does not produce probability values for Gaussian distributions. In such cases, probabilities were generated in Statistix (Version 8; Analytical Software, 2003).

The field survey results were analysed in a series of generalized linear models ('glm' command in R) for each combination of non-target species and seed predator species, with *C. nutans* presence as a fixed effect and year as a covariate; for *C. vulgare*, site was another fixed effect. Site classifies *C. nutans* presence/absence for *C. pycnocephalus* and *C. tenuiflorus* (and partially for *C. vulgare*) and could therefore not be tested in these cases; thus, Banks Peninsula and Culverden sites may vary not only in *C. nutans* presence/absence, but also in other biotic and abiotic factors which could be confounded with *C. nutans* presence/absence, and which were not estimated in the survey. This limitation was known at the time of

surveying; survey sites were selected for their species composition, and were consequently restricted by species distribution. Therefore, interpretation of the survey results is limited.

All analyses (both for the experiment and the survey) were carried out on each non-target species separately.

5.2.5 Study limitations

Several limitations of the study system have to be acknowledged:

1. The target species, *C. nutans*, is not successfully controlled by the introduced biocontrol agents in New Zealand; therefore there was no reason to expect great impact of these agents on less preferred thistle species.
2. Two of the non-target species, *C. pycnocephalus* and *C. tenuiflorus*, were only rarely found growing in proximity to *C. nutans*. This could reflect exclusion of either species from certain habitats by biocontrol interactions, but it could also reflect different habitat requirements. These species reportedly grew in association in New Zealand in the past (Jessep, 1989a), but there are no quantitative past or present data to determine whether there has been a change in the degree of co-occupancy since the introduction of biocontrol agents.
3. All four plant species selected for this study are weeds in New Zealand, with well established and widespread populations; none is a sleeper weed.
4. Regional Pest Management Strategy imposes a ban on any action that could spread *C. nutans*. Consequently, the experimental manipulation options were limited.

5.3 Results

5.3.1 Field experiment

All 120 plants per species of the annual non-target species, *C. pycnocephalus* and *C. tenuiflorus*, bolted successfully and produced capitula. In one replicate *C. pycnocephalus* and *C. tenuiflorus* plants grew unusually large, and in another they grew unusually small. It was suspected that inclusion of these unusual replicates in the analysis may increase the variance and reduce its power; therefore all *C. pycnocephalus* and *C. tenuiflorus* analyses were done on two datasets – one that includes the extreme replicates and one that excludes them; the analyses for the reduced dataset (excluding the extreme replicates; 80 plants per species) are presented herein, while the parallel analyses for the dataset which includes all six replicates are provided in Appendix B. In addition, capitulum-level variables that were measured in the field were found significant in many cases; therefore inclusion of capitula from the bulk collections (which did not have capitulum-level information) did not improve the analysis.

Bulk collections were therefore not included (with the exception of the proportion tests, where no covariates, at the capitulum level or other, could be included in the first place). Only 28 *C. vulgare* plants bolted successfully and therefore, although they too followed the pattern of extreme replicates, no analysis was done that excluded these. Information on some plant growth variables for the three non-target species, as well as for *C. nutans* is presented in Table 5.1.

Table 5.1: Raw mean, standard error, range and median of the total number of capitula produced per plant over the flowering season, peak plant height, and capitulum diameter of the four species in the system, measured from the experimental plants.
Based on 120 *C. pycnocephalus* plants, 120 *C. tenuiflorus* plants, 90 *C. nutans* plants and 28 *C. vulgare* plants which bolted and produced capitula.

		Peak capitula per plant	Peak plant height (rounded to the nearest mm)	Capitulum diameter (mm)
C. pycnocephalus	Mean (SE)	128.9 (9.1)	900 (22)	7.23 (0.99)
	Range	2 - 683	143 - 1400	2.55 - 11.95
	Median	100.5	915	7.32
C. tenuiflorus	Mean (SE)	159.2 (18.4)	802 (24)	5.66 (0.02)
	Range	10 - 1723	225 - 1423	1.88 - 10.03
	Median	96.5	816	5.60
C. vulgare	Mean (SE)	13.0 (2.0)	440 (32)	16.80 (0.22)
	Range	2 - 49	150 - 830	6.47 - 27.07
	Median	10.0	452	17.26
C. nutans	Mean (SE)	47.5 (6.3)	737 (30)	19.79 (0.09)
	Range	2 - 317	195 - 1485	4.30 - 48.78
	Median	23.0	746	19.81

The experimental plot was naturally populated with biocontrol agents. Both *R. conicus* and *U. solstitialis* appeared independently and attacked *C. nutans*. Only *R. conicus* attacked the non-target species; no *U. solstitialis* attack occurred on any species other than *C. nutans* except for a single *C. vulgare* capitulum, so no analysis was performed on *U. solstitialis* in the experient.

Rhinocyllus conicus attack on *C. pycnocephalus* and *C. tenuiflorus* dropped sharply as soon as *C. nutans* buds were starting to form, around mid December (Table 5.2). Thus, the data set as a whole is highly zero-inflated, and any effects are masked. It seemed irrational to perform complex analyses on the zero-inflated datasets, and therefore, analysis is only shown for capitula that were collected on 13 December 2005 or earlier. *Cirsium vulgare* flowers simultaneously with *C. nutans*, thus the entire dataset was analysed for this non-target species. Three plants of each *C. pycnocephalus* and *C. tenuiflorus* did not ripen any capitula before 13 December, which reflects in the sample size for some of the analyses to follow.

Table 5.2: Distribution of *R. conicus* attack on *C. pycnocephalus* and *C. tenuiflorus* capitula before and after *C. nutans* become available (13 December 2005). The change in proportion attacked over time was significant for both *C. pycnocephalus* ($\chi^2 = 555.6$, df = 1, $P < 0.001$) and *C. tenuiflorus* ($\chi^2 = 377.8$, df = 1, $P < 0.001$).

	C. pycnocephalus			*C. tenuiflorus*		
	Collected	Attacked	% attack	Collected	Attacked	% attack
Until 13 Dec. (inclusive)	1545	632	40.9	1502	457	30.4
After 13 Dec.	3285	214	6.5	3303	194	5.9
Total	4830	846		4805	651	

Two non-target species exhibited a significantly higher probability of attack by *R. conicus* in the presence of *C. nutans*: 55.0% (back transformed fitted mean) of *C. pycnocephalus* capitula were attacked in the presence of *C. nutans*, and 38.2% in its absence (Table 5.3a); 7.6% (back transformed fitted mean) of *C. vulgare* capitula were attacked in the presence of *C. nutans*, and only 0.61% in its absence (Table 5.3c). The attack on *C. tenuiflorus* was marginally non-significantly higher in the presence of *C. nutans* (35.9%, back transformed fitted mean; Table 5.3b) than in its absence (26.9%).

Table 5.3: Difference in probability (logit transformed) of capitulum attack by *R. conicus* in the presence and absence of *C. nutans* in (**a**) *C. pycnocephalus*, (**b**) *C. tenuiflorus* and (**c**) *C. vulgare*: generalized linear mixed effects models with a binomial distribution and with plants, plots and blocks as grouping factors.

(a). *C. pycnocephalus*. Based on 972 capitula from 77 plants. Intercept: *C. nutans* absent, primary cluster position.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.481	0.229	-2.101	0.036
C. nutans present	1	0.686	0.320	2.147	0.032
External diameter	1	0.251	0.080	3.143	0.002
Cluster position - secondary	1	-1.094	0.207	-5.276	<0.001
Leaf length at the time of bolting	1	-0.459	0.175	-2.616	0.009
Plant height	1	0.488	0.208	2.350	0.019
Sum of clusters on the plant	1	2.506	0.590	4.250	<0.001
Sum of capitula on the plant	1	-1.758	0.611	-2.875	0.004

(b). *C. tenuiflorus*. Based on 885 capitula from 77 plants. Intercept: *C. nutans* absent.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.998	0.172	-5.812	<0.001
C. nutans present	1	0.417	0.236	1.764	0.078
External diameter	1	0.128	0.084	1.522	0.128
Capitulum height	1	-0.501	0.111	-4.501	<0.001
Sum of capitula in the cluster	1	0.416	0.083	5.024	<0.001
Plant height	1	0.708	0.130	5.443	<0.001

(c). *C. vulgare*. Based on 305 capitula from 28 plants. Intercept: *C. nutans* absent, bolted by 6 October 2005.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-5.100	0.924	-5.521	<0.001
C. nutans present	1	2.601	0.801	3.249	0.001
Leaf length at the time of bolting	1	-2.943	0.861	-3.419	0.001
Plant height	1	5.194	1.451	3.580	<0.001
Bolted between 6 Oct. and. 21 Oct.	1	2.098	1.437	1.460	0.144
Bolted between 21 Oct. and. 16 Nov.	1	-2.611	0.874	-2.989	0.003
Bolted between 16 Nov. and. 6 Dec	1	3.568	1.670	2.137	0.033

In all three non-target species there were cases of multiple *R. conicus* attack signs per capitulum. *Carduus pycnocephalus* capitula received 0.78 (back transformed fitted mean) signs per capitulum in the presence of *C. nutans*, significantly higher than in its absence (0.38; Table 5.4a). Similarly, *C. vulgare* capitula received significantly more (however very low) attack signs in the presence of *C. nutans* (0.061 signs per capitulum; back transformed fitted mean) than in its absence (0.007; Table 5.4c). The numbers of attack signs per capitulum in *C.*

vulgare are somewhat underestimated, since eggs and penetration holes were not counted, and larval development appears to under-represent initial oviposition. In *C. tenuiflorus* the effect was marginally non significant (Table 5.4b), with 0.44 (back transformed fitted mean) attack signs per capitulum in the presence of *C. nutans* and 0.31 in its absence (Table 5.4b).

Table 5.4: Difference in the number of *R. conicus* attack signs per capitulum (log transformed) in the presence and absence of *C. nutans* in (**a**) *C. pycnocephalus*, (**b**) *C. tenuiflorus* and (**c**) *C. vulgare*: generalized linear mixed effects models with a Poisson distribution and with plants, plots and blocks as grouping factors.
(a). *C. pycnocephalus*. Based on 972 capitula from 77 plants. Intercept: *C. nutans* absent, primary cluster position.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.965	0.226	-4.275	<0.001
C. nutans present	1	0.703	0.314	2.240	0.025
External diameter	1	0.188	0.049	3.835	<0.001
Cluster position - secondary	1	-0.584	0.127	-4.595	<0.001
Sum of clusters on the plant	1	2.023	0.397	5.099	<0.001
Sum of capitula on the plant	1	-1.432	0.389	-3.681	<0.001

(b). *C. tenuiflorus*. Based on 885 capitula from 77 plants. Intercept: *C. nutans* absent.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-1.174	0.138	-8.500	<0.001
C. nutans present	1	0.356	0.186	1.912	0.056
External diameter	1	0.100	0.059	1.691	0.091
Capitulum height	1	-0.352	0.068	-5.165	<0.001
Sum of capitula in the cluster	1	0.254	0.054	4.695	<0.001
Plant height	1	0.442	0.091	4.864	<0.001

(c). *C. vulgare*. Based on 305 capitula from 28 plants. Intercept: *C. nutans* absent, bolted by 6 October 2005.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-4.937	0.720	-6.862	<0.001
C. nutans present	1	2.128	0.541	3.933	<0.001
Leaf length at the time of bolting	1	-2.694	0.604	-4.460	<0.001
Plant height	1	4.715	0.966	4.883	<0.001
Bolted between 6 Oct .and 21 Oct.	1	2.621	1.039	2.524	0.012
Bolted between 21 Oct. and 16 Nov.	1	-2.187	0.622	-3.519	<0.001
Bolted between 16 Nov. and 6 Dec.	1	3.257	1.424	2.287	0.022

Only for *C. vulgare* was *C. nutans* presence *per se* a predictor for the level of damage within the capitulum (Table 5.5c). The number of attack signs by *R. conicus* was a significant predictor of damage level for all three non-target species (Table 5.5): for all three species the level of damage increased with more attack signs; for *C. vulgare* the level of damage increased also in the presence of *C. nutans*, compared to in its absence. Of course the number of attack signs was affected by *C. nutans* presence, giving an indirect effect of presence on level of damage.

Table 5.5: Difference in the cumulative proportion (logit transformed) of (**a**) *C. pycnocephalus*, (**b**) *C. tenuiflorus* and (**c**) *C. vulgare* capitula (of the total processed) exhibiting increasing *R. conicus* feeding damage rank in the presence and absence of *C. nutans*: proportional odds (cumulative logit) models. The model is interpreted as a series of regression lines on a logit scale (one for each cut point); to model capitula in the absence of *C. nutans*, the slope term for '*C. nutans* present' is omitted.

(a). *C. pycnocephalus*. Based on 973 capitula.

Fixed effects

	df	Estimate	Std. Error	t value	*P*
C. nutans present	1	0.039	0.23	0.17	0.865
Sum of *R. conicus* signs	1	1.348	0.10	13.02	<0.001
Cluster position - secondary	1	-0.615	0.32	-1.94	0.053
Leaf length at the time of bolting	1	-0.400	0.15	-2.70	0.007
Sum of capitula on the plant	1	0.730	0.22	3.36	0.001
Sum of capitula in the plot	1	-0.809	0.29	-2.83	0.005
Average plot height	1	0.641	0.23	2.76	0.006

Intercepts for cut points of damage categories

	df	Estimate	Std. Error	t value	*P*
No damage (0.180, 0.139)[1]	1	2.186	0.178	12.298	<0.001
Little damage (0.169, 0.135)	1	2.270	0.180	12.635	<0.001
Medium damage (0.120, 0.090)	1	2.875	0.195	14.768	<0.001

Table 5.5 (contd)
(b). *C. tenuiflorus*. Based on 869 capitula.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
C. nutans present	1	0.062	0.24	0.26	0.795
Sum of *R. conicus* signs	1	1.980	0.14	14.51	<0.001
External diameter	1	-0.222	0.11	-2.12	0.034
Plant height	1	0.438	0.12	3.66	<0.001
Bolted between 26 Sep. and 6 Oct.	1	-0.147	0.24	-0.62	0.535
Bolted between 6 Oct. and 21 Oct.	1	-0.169	0.36	-0.47	0.638
Bolted between 21 Oct. and 6 Dec.	1	-0.716	0.96	-0.75	0.453

Intercepts for cut points of damage categories					
	df	Estimate	Std. Error	t value	*P*
No damage (0.231, 0.230)[1]	1	1.772	0.217	8.16	<0.001
Little damage (0.216, 0.219)	1	1.928	0.219	8.80	<0.001
Medium damage (0.179, 0.170)	1	2.434	0.227	10.71	<0.001

(c). *C. vulgare*. Based on 305 capitula.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
C. nutans present	1	2.02	0.94	2.14	0.0330
Sum of *R. conicus* signs	1	1.27	0.34	3.74	<0.001
Plant height	1	1.61	0.73	2.21	0.0279
Bolted between 6 Oct. and 21 Oct.	1	-5.31	3.38	-1.57	0.1170
Bolted between 21 Oct. and 16 Nov.	1	-3.62	1.35	-2.69	0.0080
Bolted between 16 Nov. and 6 Dec.	1	-0.17	1.60	-0.11	0.9120

Intercepts for cut points of damage categories					
	df	Estimate	Std. Error	t value	*P*
No damage (0.078, 0.037)[1]	1	3.77	0.76	4.96	<0.001
Little damage (0.034, 0.010)	1	5.82	1.05	5.52	<0.001
Medium damage (0.017, 0.005)	1	8.29	1.92	4.30	<0.001

[1]Numbers in parentheses beside each cut point for damage categories are the cumulative proportions of capitula exhibiting higher damage rating than that cut point in the presence and absence of *C. nutans* respectively.

The presence of attack signs does not necessarily ensure attack success (i.e. feeding damage to receptacle tissue). Most attacks on *C. pycnocephalus* and *C. tenuiflorus* resulted in no damage to the attacked capitulum, whether in the presence of *C. nutans* or in its absence (Table 5.6a & b). The percentage of damaged capitula of the total examined ranged between ~16% early in the season to ~3% later in the season for *C. pycnocephalus*, and between ~21% to ~3% for *C. tenuiflorus*. In *C. vulgare*, the majority of attacked capitula exhibited some damage (Table 5.6c), but this is not surprising, because *C. vulgare* capitula are larger and

would have received a higher number of eggs per attacked capitulum, hence a higher likelihood of showing some degree of damage, even if only very low, as a result of attack. The raw numbers of collected, attacked and damaged capitula are provided in Appendix B.

Table 5.6: Difference in the percentage of non-target species capitula attacked by *R. conicus* (of the total processed) and the proportion of capitula exhibiting actual *R. conicus* feeding damage (of those that got attacked) early in the season (before 13 December; for *C. pycnocephalus* and *C. tenuiflorus*) and over the entire season (for *C. vulgare*), in the presence and absence of *C. nutans*. For *C. pycnocephalus* and *C. tenuiflorus*, the percentages of damaged capitula of those attacked are also compared between early and late season (regardless of *C. nutans* presence). Sub-samples from the bulk collections (for *C. pycnocephalus* and *C. tenuiflorus*) were either excluded or included.

(a). *C. pycnocephalus*.

	Bulk collections excluded			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	45.6 (450)	39.5 (205)	Early season	37.6 (410)
Early season, *C. nutans* absent	39.0 (526)	35.6 (205)	Late season	53.3 (152)
χ^2	4.31	0.67		11.28
P	0.038	0.415		0.001
	Bulk collections included			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	40.3 (682)	40.7 (275)	Early season	38.3 (617)
Early season, *C. nutans* absent	36.9 (927)	36.3 (342)	Late season	50.5 (313)
χ^2	1.95	1.29		12.72
P	0.162	0.256		<0.001

(b). *C. tenuiflorus*.

	Bulk collections excluded			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	35.4 (458)	65.4 (162)	Early season	66.2 (308)
Early season, *C. nutans* absent	34.2 (430)	67.1 (146)	Late season	59.2 (164)
χ^2	0.14	0.10		2.33
P	0.711	0.754		0.127
	Bulk collections included			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	32.3 (746)	63.1 (241)	Early season	66.5 (486)
Early season, *C. nutans* absent	28.6 (860)	69.8 (245)	Late season	63.4 (265)
χ^2	2.59	2.47		0.71
P	0.108	0.116		0.399

Table 5.6 (contd.)
(c). *C. vulgare*. The difference in percent damaged of attacked in the presence and absence of *C. nutans* could not be tested due to small sample size.

	% attacked (N)	% damaged of attacked (N)
C. nutans present	8.7 (115)	90.0 (10)
C. nutans absent	3.7 (190)	85.7 (7)
χ^2	3.35	
P	0.067	

Tables 5.7 & 5.8 and Figure 5.2 present the simple regressions relating seed production to capitulum external diameter in the three non-target species, in the absence of *R. conicus* attack. Regressions using receptacle diameter and receptacle area were attempted as well, but external diameter (presented) gave the best correlation coefficient and was therefore selected for further seed output estimates. Increased feeding damage by *R. conicus* inflicted increased seed loss (Figure 5.3). The proportions of seed lost per capitulum in each damage category were incorporated into the seed production and loss estimates per plant.

Table 5.7: Parameter estimates for regression components for the relationships between seed counts (log transformed) and external capitulum diameter in mm in **(a)** *C. pycnocephalus*, **(b)** *C. tenuiflorus* and **(c)** *C. vulgare*: mixed effects models with poisson distribution and with plants as a grouping factor.

(a) *C. pycnocephalus*. Based on 2025 capitula from 116 plants

Fixed effects					
	df	Estimate	Std. error	z value	*P*
Intercept	1	-0.328	0.051	-6.440	<0.001
External diameter	1	0.353	0.006	57.750	<0.001

(b) *C. tenuiflorus*. Based on 706 capitula from 100 plants

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.837	0.090	-9.270	<0.001
External diametre	1	0.489	0.013	38.640	<0.001

(c) *C. vulgare*. Based on 220 capitula from 28 plants

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	1.882	0.082	22.960	<0.001
External diameter	1	0.150	0.003	58.720	<0.001

Table 5.8: Simple regression components for seeds per capitulum as a function of external diameter for un-attacked non-target species capitula, accounting for variation between plants.

	N - capitula	N - plants	Intercept	95% CI	Slope	95% CI
C. pycnocephalus	2025	116	0.72	0.65-0.80	1.42	1.41-1.44
C. tenuiflorus	706	100	0.43	0.36-0.52	1.63	1.57-1.67
C. vulgare	220	28	6.57	5.59-7.71	1.16	1.16-1.17

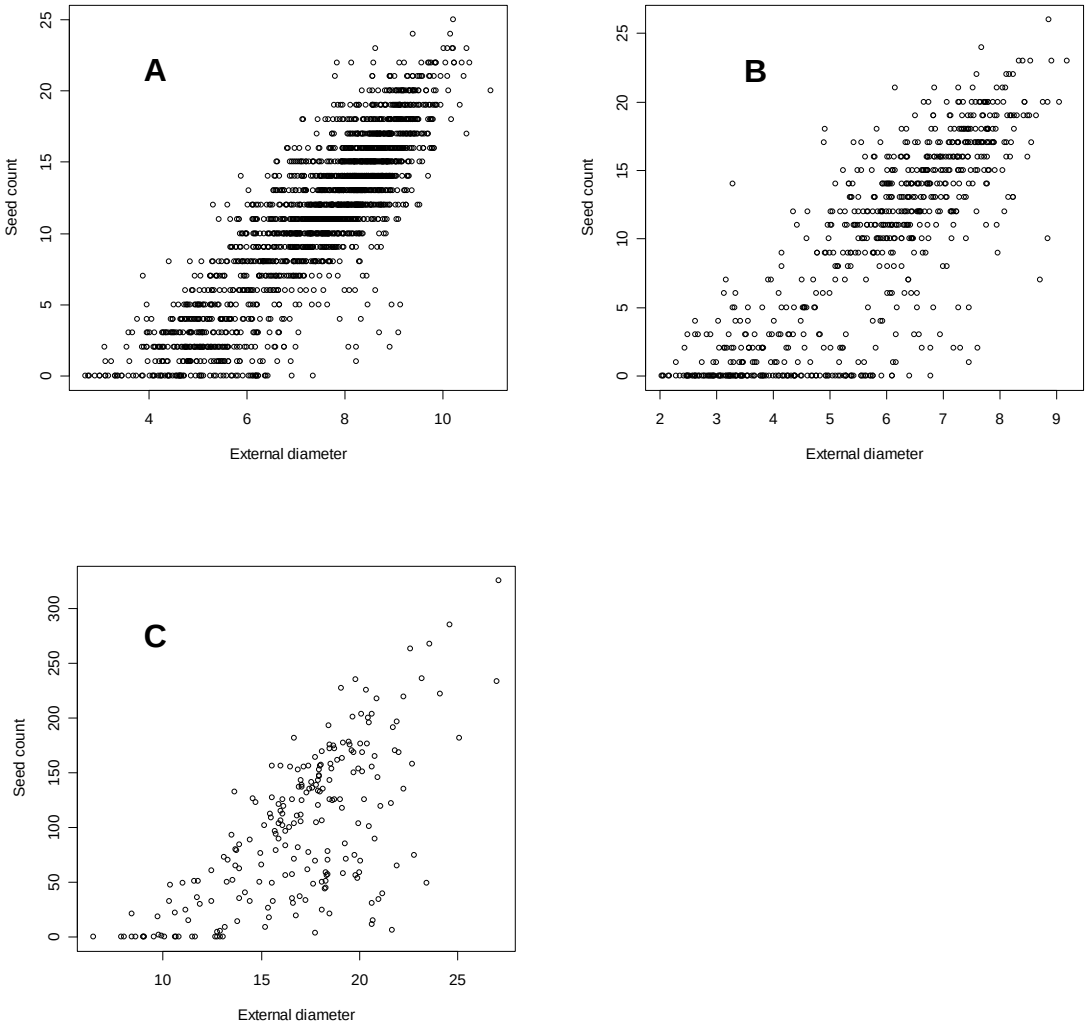

Figure 5.2: Relationships between capitulum diameter (mm) and seed count in un-attacked capitula of (**A**) *C. pycnocephalus*, (**B**) *C. tenuiflorus* and (**C**) *C. vulgare*.

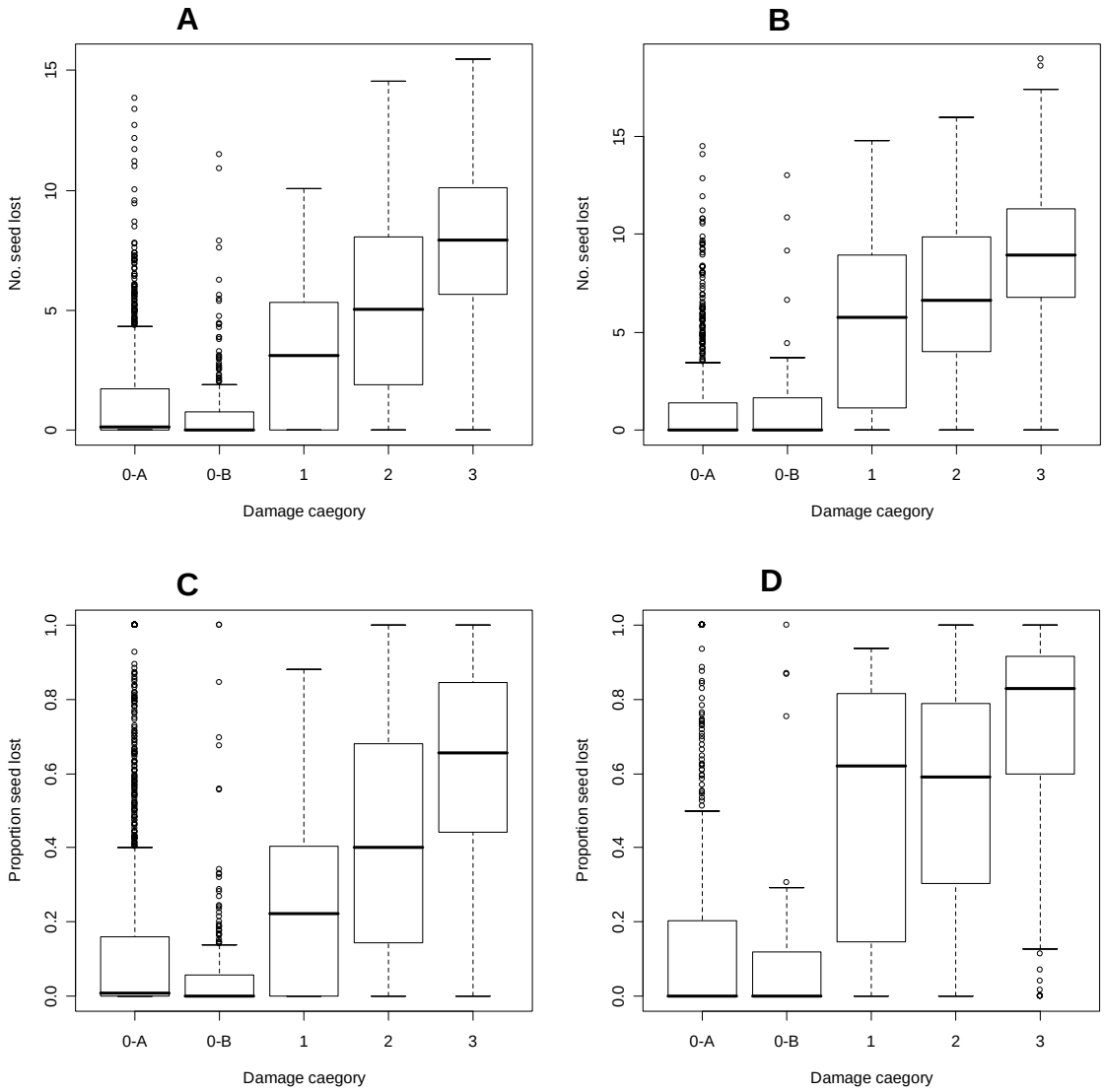

Figure 5.3: Median seed loss per capitulum (A & B) and median proportion of seed lost per capitulum (C & D) in response to *R. conicus* damage in *C. pycnocephalus* (A & C) and *C. tenuiflorus* (B & D). Damage category 0-A is for un-attacked (hence un-damaged) capitula; 0-b is for attacked yet un-damaged capitula.

Proportional seed loss analyses were done on the dataset which included all six replicates because of the small sample size (23 plants per species; this would have reduced to only 16 plants per species in the dataset which excluded the two extreme replicates). There was no significant difference in *C. pycnocephalus* proportional seed loss to *R. conicus* in the presence and absence of *C. nutans* (Table 5.9). Interestingly, the proportional seed loss in *C. tenuiflorus* was smaller in the presence of *C. nutans* (Table 5.9b), which is opposite to the expected, but the effect was non-significant. This is inconsistent with the results thus far, which indicated higher probability of attack, more attack signs per capitulum and (therefore) higher damage in the presence of *C. nutans*. However, the proportional seed loss tests were done only on plants that had all their capitula individually harvested (one plant per species per plot), which is a much smaller dataset than that used in the earlier analyses. Proportional seed

loss tests could not be performed on *C. vulgare*, because there were not enough capitula to construct a relationship between *R. conicus* feeding damage and seed output.

Table 5.9: Difference in proportion of seeds lost to *R. conicus* in (**a**) *C. pycnocephalus* and (**b**) *C. tenuiflorus* plants in the presence and absence of *C. nutans*: mixed effects models with binomial distribution and with blocks as a grouping factor. All replicates were included. (a). *C. pycnocephalus*. Based on 23 plants. Intercept: *C. nutans* absent, bolted before 26 September 2005.

Fixed effects					
	df	Estimate	Std. error	z value	*P*
Intercept	1	-1.763	0.022	-79.380	<0.001
C. nutans present	1	0.044	0.038	1.150	0.250
Bolted between 26 Sep. and 6 Oct.	1	-0.162	0.053	-3.050	0.002

(b). *C. tenuiflorus*. Based on 23 plants. Intercept: *C. nutans* absent.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-1.206	0.025	-48.430	<0.001
C. nutans present	1	-0.050	0.035	-1.420	0.154
Sum of capitula on the plant	1	-0.059	0.022	-2.660	0.008
Plant height	1	0.067	0.030	2.260	0.024

5.3.2 Field survey

In the field survey, *R. conicus* attack on the non-target species was generally low (Figures 5.4, 5.5 & 5.6), and comparable to the level experienced at the experiment. Attack by *Urophora* sp. on a non-target species was only recorded on one occasion, in Culverden; this is likely to be *U. solstitialis* and not *U. stylata*, because the attack was recorded from *C. tenuiflorus*, while no attack was recorded from *C. vulgare* (which is present in that area). *Urophora solstitialis* attack on *C. vulgare* was not expected (and was not seen). A series of generalized linear models shows the attack was significantly higher in the presence of *C. nutans* than in its absence, except for *C. vulgare*, where the effect was marginally non-significant (Table 5.10c). The level of attack on *C. nutans* (where present) may explain the non-significant effect in the case of *C. vulgare*: 86.4 and 62.0% of *C. nutans* capitula were attacked in Culverden area in 2005-06 and 2006-07 respectively; however, in Rakaia Gorge area where *C. nutans* and *C. vulgare* co-occurred only 17.6% of the *C. nutans* were attacked in 2005-06, compared to 88.0% in 2006-07. Thus it seems that *R. conicus* was uncommon at Rakaia Gorge in the first season, probably reducing the non-target impact on *C. vulgare*. The raw numbers of attacked and un-attacked non-target capitula are provided in Appendix B. At any rate however, the

models need to be treated cautiously, due to the confounding effects of regions and *C. nutans* presence/absence.

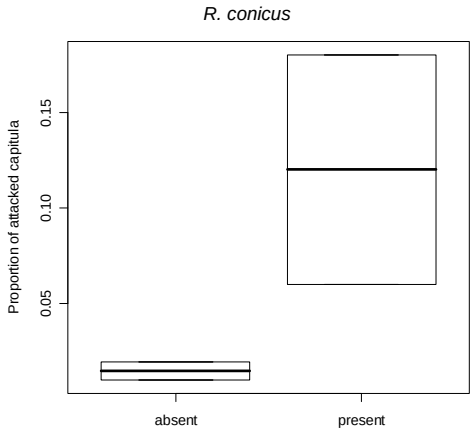

Figure 5.4: Raw median proportion of *R. conicus* attack on *C. pycnocephalus* in the presence (Culverden) and absence (Banks Peninsula) of *C. nutans* in field surveys in 2005-06 and 2006-07.

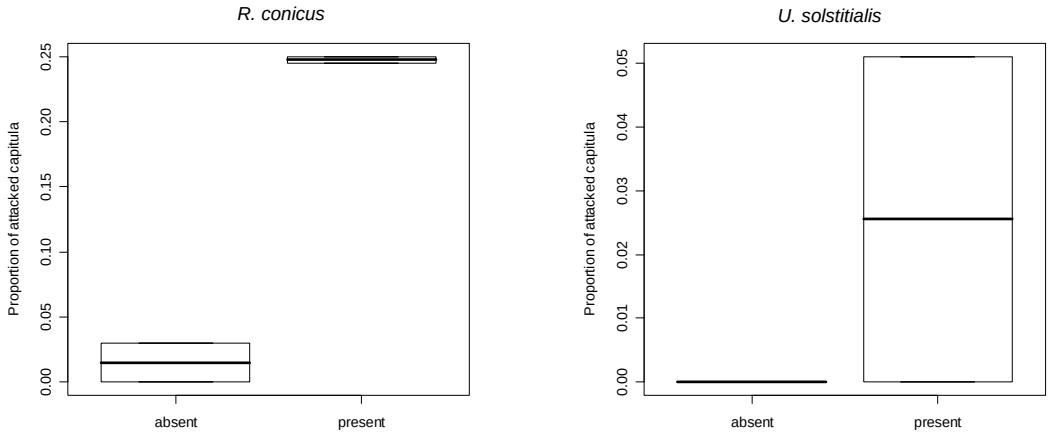

Figure 5.5: Raw median proportion of biocontrol agents attack on *C. tenuiflorus* in the presence (Culverden) and absence (Banks Peninsula) of *C. nutans* in field surveys in 2005-06 and 2006-07.

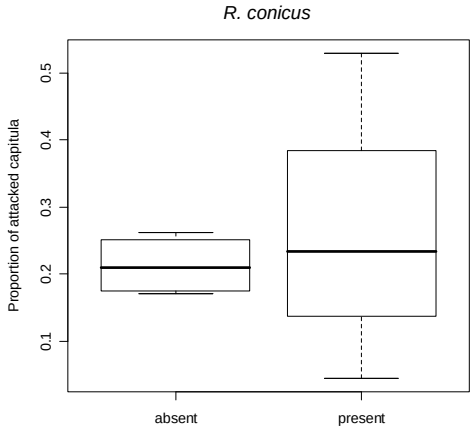

Figure 5.6: Raw median proportion of *R. conicus* agents attack on *C. vulgare* in the presence (Culverden and Rakaia Gorge) and absence (Banks Peninsula and Rakaia Gorge) of *C. nutans* in field surveys in 2005-06 and 2006-07.

Table 5.10: ANOVA tables for the effects of *C. nutans* presence and year on biocontrol agent attack on (**a**) *C. pycnocephalus*, (**b**) *C. tenuiflorus* and (**c**) *C. vulgare* in field surveys in 2005-06 and 2006-07: generalized linear models.

(a). *C. pycnocephalus*

R. conicus	df	Deviance	Resid. df	Resid. dev.	F value	*P*
Null	401	197.978				
C. nutans presence	1	19.995	400	177.983	19.995	<0.001
Year	1	5.187	399	172.796	5.187	0.023
U. solstitialis						
Null	401	0				
C. nutans presence	1	0	400	0	0	1
Year	1	0	399	0	0	1
Either						
Null	401	197.978				
C. nutans presence	1	19.995	400	177.983	19.995	<0.001
Year	1	5.187	399	172.796	5.187	0.023

Table 5.10 (contd.)
(b). *C. tenuiflorus*

R. conicus	df	Deviance	Resid. df	Resid. dev.	F value	*P*
Null	399	309.109				
C. nutans presence	1	56.317	398	252.793	56.317	<0.001
Year	1	0.160	397	252.632	0.160	0.689
U. solstitialis						
Null	399	53.758				
C. nutans presence	1	7.097	398	46.661	7.097	0.008
Year	1	7.165	397	39.496	7.165	0.007
Either						
Null	399	323.970				
C. nutans presence	1	62.710	398	261.260	62.708	<0.001
Year	1	1.030	397	260.230	1.033	0.309

(c). *C. vulgare*

R. conicus	df	Deviance	Resid. df	Resid. dev.	F value	*P*
Null	791	870.41				
C. nutans presence	1	3.04	790	867.37	3.042	0.081
Year	1	1.71	789	865.66	1.711	0.191
Site	2	28.26	787	837.40	14.132	<0.001
Site x Year	2	31.49	785	805.90	15.747	<0.001

5.4 Discussion

Three non-target thistle species with different life histories and different phenology in my study suffered associational susceptibility to the shared herbivore, *R. conicus*, when in the presence of the preferred host, *C. nutans*. At the experiment, all three non-target species experienced increased probability of attack, higher numbers of attack signs per capitulum and increased damage in the presence of *C. nutans*. In *C. vulgare*, increased damage was inflicted by *C. nutans* presence *per se*, whereas in *C. pycnocephalus* and *C. tenuiflorus*, the effect was expressed via the number of attack signs per capitulum. At the survey sites, all three species experienced higher probability of attack in regions hosting *C. nutans* compared to regions where it is absent. Thus the hypothesis that the non-target species are attacked and damaged more in the presence of the target species is supported both at the small spatial scale of the experiment and at the larger spatial scale of the survey.

Associational susceptibility forms the foundations for multi targeting, and it is important to understand the mechanisms driving it. Theory predicts associational susceptibility should occur as a result of a shortage of the preferred host, or as a consequence of the herbivores increased attraction to high-quality patches, where the preferred host is present (Hjalten *et al.*, 1993). Otherwise, associational susceptibility can occur when the

herbivore aggregates at high density on the preferred host, and as the preferred host is depleted, the herbivore 'spills over' to the secondary host (White & Whitham, 2000; Blossey *et al.*, 2001).

What mechanisms drove associational susceptibility in the current study? Overexploitation of *C. nutans* and spillover effect cannot explain the attack on *C. pycnocephalus* and *C. tenuiflorus* early in the season, simply because *C. nutans* was not yet available at that time. *Carduus pycnocephalus* and *C. tenuiflorus* are not preferred hosts for *R. conicus* (they flower early in the season, when *R. conicus* is active, yet, even at peak attack, no more than 40% of capitula were attacked at the experiment, as opposed to 100% of earliest *C. nutans* capitula; see Chapter 3). In addition, *C. pycnocephalus* and *C. tenuiflorus* support fewer and smaller *R. conicus* progeny (Chapter 6). Similarly, *C. vulgare* is not a preferred host and, despite similarity in size to *C. nutans*, it receives far less attack and does not support *R. conicus* development as does *C. nutans* (Rees, 1977; pers. obs.). In the field survey, *C. pycnocephalus* and *C. tenuiflorus* populations growing in the absence of *C. nutans* exhibited only 0-3% attack (Figures 5.4 & 5.5; Appendix B). It is possible that *R. conicus* is not attracted to and/or does not often accept patches only hosting these species. The effect was not as strong in the experiment, where the small spatial scale did not allow for patches to be far enough from *C. nutans*. *Cirsium vulgare* populations that were isolated from *C. nutans* in the survey did, however sustain small *R. conicus* populations. Hardly anything is known about *R. conicus* attraction to host plants; nevertheless, recent work confirmed olfactory behaviour of the weevil, in relation to *C. nutans* bolting plants, as well as to rosettes (Sezen, 2007). This would mean that *C. nutans* presence could have attracted *R. conicus* to my experimental site, despite *C. nutans* only being at the rosette stage.

In the experiment, *R. conicus* has demonstrated long distance flight abilities to naturally colonise the experimental plants, beyond expectation. It is unclear exactly what was the distance flown to colonize the site, but it is estimated to be several kilometres; the site was situated within a township, several km away from farming areas, in a region that only suffers low *C. nutans* densities (Chapter 7). Considering these flight abilities, *R. conicus* was in a position to reject the experimental patch and search further. It is suggested here that the weevils accepted the patch owing to *C. nutans* presence. Moreover, compared to the potentially great distance flown to reach the experimental site, the distances between plots hosting *C. nutans* and plots not hosting it were negligible; still, associational susceptibility was inflicted on the non-target species significantly more in the presence of *C. nutans*, suggesting attraction even at this small scale.

It is suggested that in addition to attracting *R. conicus* to the site, the presence of *C. nutans* enhanced associational susceptibility on the non-target species by enhancing acceptance of the patch by the weevil - while the weevils were at the site, awaiting *C. nutans* flowering to commence, *C. pycnocephalus* and *C. tenuiflorus* were continuously producing new capitula, more of which were attacked than would be expected if they were not growing in association with *C. nutans*.

At the point in time when *C. nutans* just started flowering, the first few capitula were severely attacked (Chapter 3); at that time, shortage of the preferred host (overexploitation) may explain why oviposition on *C. pycnocephalus* and *C. tenuiflorus* did not entirely cease. However, attack on *C. vulgare* occurred when *C. nutans* capitula were not exploited in their entirety, and it is possible that aggregation of *R. conicus* on *C. nutans* plants led to spillover attack on *C. vulgare* plants in these plots. A recent *R. conicus* - thistle system has demonstrated associational susceptibility without the preferred host *C. nutans* getting over-exploited (Russell *et al.*, 2007).

Multi-targeting in general, and multi-targeting of thistles in particular, gains good support from my data in that three non-target host species, which exhibit different life histories and different phenologies, suffered associational susceptibility in the presence of a preferred host. Yet, increased attack and increased damage alone are insufficient to make multi-targeting work; an effect on the non-targets growth or performance, and a population level effect are required. In my study, the increased attack and increased damage on *C. pycnocephalus* and *C. tenuiflorus* in the presence of *C. nutans*, did not translate to a significant proportional seed loss (and the effect on *C. vulgare* was non-measurable). This is not surprising, considering the low attack level over the entire season (overall 17.5 and 13.5% of *C. pycnocephalus* and *C. tenuiflorus* capitula respectively; calculated from Table 5.2), and considering that only a low proportion of capitula that were attacked suffered damage. In addition, even damaged capitula bore some healthy seeds in many cases. Even if a significant proportional seed loss had been demonstrated, an adverse effect on seedling recruitment (a population level effect) would still had to have been confirmed. An adverse effect on the non-target species at the population level could be expected if several conditions are met, one of which is that the non-target species has a low intrinsic growth rate (and hence does not replenish loss easily), another being that the shared predator (herbivore in this case) attacks both the target and the non-target species at high rates (Holt & Hochberg, 2001). These conditions were not met in the system selected for this study: all four weed species in both the experiment and the field survey are well established weeds (i.e., exhibit high intrinsic growth rate; none is a sleeper weed); moreover, the biocontrol agent, *R. conicus*, does not decrease

populations of its preferred host *C. nutans* successfully, nor does it overexploit it; hence it would not be expected to inflict population level effects on the less preferred, well established non-target hosts. Unsurprisingly, attack rates were not found in my study that could be translated to the population level. Interestingly, in Nebraska, despite the preferred host *C. nutans* not getting over-exploited (Russell *et al.*, 2007) it is suggested, from circumstantial evidence, that *R. conicus* may have an adverse effect on the population of a native North American thistle (Louda & Arnett, 2000); however, this effect is not explained by associational susceptibility, since *C. nutans* and the native thistle do not overlap spatially (Rand & Louda, 2006). In addition, the native North American thistle population is regulated by native North American seed predators (Louda & Potvin, 1995; Guretzky & Louda, 1997), such that *R. conicus* adds further pressure, unlike in New Zealand, where no native herbivores regulate thistles.

That associational susceptibility can have a population level effect on the secondary host has been previously shown (e.g., Thomas, 1986). When the secondary host is a sleeper weed, with a low intrinsic growth rate, associational susceptibility could play a significant role in preventing it from transiting from the 'lag phase' to the invasive phase. Populations are considered to be at the 'lag phase' when population growth is slow and confined. Populations are restrained at the 'lag phases' by availability of suitable habitat and by barriers to long-distance dispersal (Cousens & Mortimer, 1995). Long-distance dispersal events may require external means, and may initially be rare. The probability to disperse from the source population may be effectively zero, until the population grows to a sufficiently high density. Thus, the species must increase in abundance locally before it is likely to disperse successfully to long-distances and colonise new regions (Cousens & Mortimer, 1995). Associational susceptibility at the 'lag phase' is likely to prove sufficient in many cases to prevent the sleeper weed from building up high local abundance required to facilitate long-distance dispersal and invasion.

5.4.1 Conclusions

This study verifies that the presence of a preferred weed species can inflict associational susceptibility on other, less preferred weed species, thus supporting the concept of multi-targeting. Associational susceptibility may trigger multi-targeting, but cannot by itself guarantee success. For multi-targeting to succeed, both the target weed and the "non-target" sleeper weed must suffer a clear adverse population effect. Thus, a multi-targeting programme is most likely to be successful when (1) the 'non-target' weed is indeed a sleeper weed and not a well established weed (so that its populations may be limited by 'spillover' herbivory);

(2) the sleeper weed, at least in part of its distribution, co-occurs with the target weed (so that associational susceptibility could arise); (3) the presence of the target weed indeed inflicts associational susceptibility on the sleeper weed; and (4) this herbivory could regulate both weeds. In addition, where the target and the sleeper co-occur, it is necessary to confirm that no direct competition between them regulates their populations; otherwise, associational susceptibility may simply balance such competitive effects (Holt & Hochberg, 2001) and neither population would decrease.

Another challenge for multi-targeting lies in the selection of biocontrol agents. Theory predicts that non-target effects of the form of associational susceptibility are most likely when the biocontrol agent has a moderate effect on the target weed: the target weed populations remain high enough to sustain large populations of the biocontrol agent, which can maintain attack on non-targets even when they are present at low density (Holt & Hochberg, 2001). These circumstances are, however, not desirable for multi-targeting in which, as in any biocontrol introduction, the agent is expected to severely, rather than moderately affect the target weed. An attractive agent for multi-targeting would be one that attacks the target as well as the sleeper weeds at a high rate. The resulting overexploitation expected under this scenario (Holt & Hochberg, 2001) would be the desirable outcome of multi-targeting. In thistles, this was suggested to occur when the root herbivore *Trichosirocalus horridus sensu* (Panzer) (for clarification see Chapter 2) encountered a mixed population of its preferred host, *Carduus thoermeri* (a synonym of *C. nutans* ssp. *leiophyllus*), and the less preferred host *Carduus acanthoides*: at low *T. horridus* densities, mainly the preferred host was utilized; but an increase in *T. horridus* density resulted in reduced densities of the preferred host, a proportional increase of the less preferred host, and subsequently, more attack on the less preferred host, to the degree that both hosts were decreasing in density (Kok, 1986).

A further challenge lies in the establishing the generality of the multi-targeting concept i.e., demonstrate systems exist to which multi-targeting could be applied. Few examples from New Zealand include: (a) the family Buddlejaceae, represented in New Zealand by five species in the genus *Buddleja*, two of which (in one genus) are weeds here and the other three are naturalised here and have been recorded as weedy elsewhere; (b) the family Asclepiadaceae, represented in New Zealand by four species in two genera. Two species are weedy here, one is naturalised here and weedy elsewhere, and the fourth is not naturalised here, but recorded as weedy elsewhere. It has to be noted though that the two non-weedy species are commonly planted in gardens to attract monarch butterflies, and may present a conflict of interests; (c) the family Caprifoliaceae is represented in New Zealand by six species and one hybrid, in three genera. One species in each of the three genera is a weed

here. Two more species and the hybrid are naturalised here and recorded as weedy elsewhere, and for the remainder species New Zealand is the first place to record naturalisation. There used to be an indigenous genus associated with this family, but it has long been moved to the family Alseuosmiaceae; (d) the tribe Eupatorieae in the family Asteraceae is represented in New Zealand by six species in five genera. Three species in two genera are weeds here, one of which is cultivated. Two more species are naturalised here and weedy elsewhere, and for the remainder species New Zealand is the first place to record naturalisation. The two weedy species of the genus *Ageratina* are under successful biological control in New Zealand, and retrospectively, it is thought that, had a biocontrol agent with a wider host range been introduced against *A. adenophora* (Spreng.) (Mexican devil weed), it is quite likely that *A. riparia* (Rigel) King & Robinson (mist flower) would not have become weedy in the first place (S. V. Fowler, pers. Comm..); (e) the family Cactaceae is represented in New Zealand by two species in one genus. Both are naturalised here, and have been recorded as weeds elsewhere. In New Zealand they are mainly restricted by cold weather, however with parts of New Zealand becoming warmer and drier, the likelihood of these cacti to increase their range is increasing. These are just several examples from New Zealand. There are potentially plenty other cases of plant groups that are not represented in the native flora and contain weedy, as well as sleeper weeds, both in New Zealand and globally.

The constant increase in the number of weeds that become naturalized and invasive poses a challenge to the traditional biological control paradigm of targeting a single weed at a time. In many cases, alien species which belong to exotic genera possess different traits to those possessed by native flora, and are hence more likely to be invasive (Rejmanek, 1999). This idea had already been expressed by Darwin (1860) (but see Duncan & Williams, 2002). Multi targeting, hence, is a timely and innovative approach that is likely to be found appropriate in a wide range of weedy arenas. Further research should focus on predicting which target weeds and agents combinations could inflict associational susceptibility on which sleeper weeds, and more importantly, when is this likely to result in population decreases in either or both the target and the sleeper weed. In the thistle biocontrol programme, it would be highly beneficial to test such predictions on a new thistle biocontrol agent that is currently being released in New Zealand, the leaf beetle *Cassida rubiginosa* Mueller.

Chapter 6[1]: Factors affecting oviposition rate in the weevil *Rhinocyllus conicus* on non-target *Carduus* spp. in New Zealand

6.1 Introduction

Rhinocyllus conicus (Froehlich) (Coleoptera: Curculionoidae), nodding (musk) thistle receptacle weevil, is known to attack different thistle species, but displays a clear preference for nodding thistle, *Carduus nutans* L. (Zwölfer & Harris, 1984). Eggs are laid on the developing flower buds, and the larvae burrow their way into the receptacle, where their feeding prevents seed formation (Zwölfer & Harris, 1984). The weevil, introduced to New Zealand in 1972 (Jessep, 1989a), was the first biocontrol agent deployed here for nodding thistle biological control.

In New Zealand, there are no native plants in the tribe Carduae, and the only crop plant belonging to the tribe, globe artichoke, is of minor economic importance (Paynter *et al.*, 2004). Thus, any thistle here if not a weed already, could potentially become one in the future; and any impact a biocontrol agent such as *R. conicus* may have on any thistle here is, therefore, desirable.

We hypothesised that since *R. conicus* is highly attracted to *C. nutans* and is less likely to leave patches of *C. nutans*, then less preferred (non-target) thistle species are more likely to be attacked by the weevil in patches they share with *C. nutans*, than in patches from which *C. nutans* is absent. We selected two species that are closely related to *C. nutans* as non-target species; they were *Carduus tenuiflorus* and *C. pycnocephalus* L., and we tested the level to which they were attacked by *R. conicus* when *C. nutans* was present in close proximity, vs. when it was absent. We also examined what plant characteristics may affect *R. conicus* oviposition rate among these species, in an attempt to separate this effect from that of *C. nutans* proximity.

6.2 Materials and Methods

Carduus pycnocephalus, *C. tenuiflorus*, and *C. nutans* plants were grown in the University of Canterbury glasshouses from seeds collected in summer 2005 around Canterbury. In late autumn 2005 they were transplanted as small rosettes to the experimental site at the Landcare Research Lincoln campus.

[1] This chapter is now in press and may be cited as (Groenteman *et al.*, in press a).

At the experimental site, non-target species rosettes were organized into plots, each containing five *C. tenuiflorus* plants, five *C. pycnocephalus* plants and five *Cirsium vulgare* plants (not discussed further) in a 3 × 5 randomised arrangement, with 0.2 m between plants and 3 m between plots, for a total of 24 plots. Plots were grouped in blocks of four plots each, with at least 5 m between blocks. Two plots in each block were randomly assigned a "*C. nutans* present" treatment, and in these, 10 *C. nutans* rosettes were planted 0.2 m apart, in two rows, 0.4 m away from the non-target plants. The experiment was designed with six blocks (replicates), with four treatments per block (*C. nutans* present/absent and cage present/absent, to control for weevil density), but the cage treatment was effectively cancelled due to difficulty obtaining sufficient weevils early enough in the season, when the non-target plants were already bolting. Weevils populated the experiment independently from the surrounding environment, and thus, *R. conicus* densities were not controlled.

The longest leaf of each rosette was measured at bolting, and plant height, number of clusters, their position, and number of capitula, were recorded fortnightly. From each plot, one plant of each non-target species was randomly selected, from which all the ripe capitula were collected fortnightly and placed individually into paper bags. The height and number of capitula in the cluster were recorded for these individually collected capitula. The receptacle diameter was measured for each capitulum and any sign of attack by *R. conicus* was recorded. For each non-target species, the difference in *R. conicus* attack signs per capitulum (log transformed) were compared between treatments (*C. nutans* present vs. absent) in mixed models with Poisson distribution using R (R Development Core Team, 2006); all different plant and capitulum variables were included in the models as covariates, and non-significant variables were excluded in backwards selection using the Chi-squared test to compare between models.

Emerging *R. conicus* adults were sexed and elytra length was measured. The length was then compared between sexes and between thistle species using an F test (which included a comparison to adults that emerged from early *C. nutans* capitula in the experiment).

6.3 Results and Discussion

Rhinocyllus conicus attack (number of attack signs per capitulum) on the non-target species did not differ between plots with and without *C. nutans* (Table 6.1). *C. pycnocephalus* was attacked more than *C. tenuiflorus* (Table 6.1).

Table 6.1: Number of *R. conicus* attack signs per capitulum (back-transformed from log-scale means with 95% CI).

	Thistle species	
Treatment	*C. tenuiflorus*	*C. pycnocephalus*
C. nutans present	0.025 (0.015-0.042)	0.11 (0.07-0.17)
C. nutans absent	0.028 (0.017-0.05)	0.10 (0.06-0.16)

On both *C. tenuiflorus* and *C. pycnocephalus*, *R. conicus* preferred larger capitula on larger plants for oviposition, and attack on these species was stronger early in the spring, decreasing towards summer (equations $A_{c.t}$ and $A_{c.p}$ below).

The equations describing the effects of the measured covariates on *R. conicus* attack (number of signs per capitulum) for *C. tenuiflorus* ($A_{c.t}$) and *C. pycnocephalus* ($A_{c.p}$) are, respectively:

$$A_{c.t} = e^{(-3.6 - 1.01\times\text{day} - 0.48\times\text{c. height} + 0.46\times\text{p. height} + 0.35\times\text{diameter} + 0.09\times\text{cl. size} + 0.002\times\text{cap})}$$

$$A_{c.p} = e^{(-2.24 - 2.97\times\text{tertiary} - 0.90\times\text{secondary} + 0.52\times\text{diameter} - 0.44\times\text{day} + 0.29\times\text{p. height} - 0.11\times\text{c. height} + 0.006\times\text{cap})};$$

where "day" is day of the year on which the capitulum was collected, "c. height" is height of the individual capitulum (mm), "p. height" is maximum height of the plant at the time of collection (mm), "diameter" is external diameter of the individual capitulum (mm), "cl.size" is cluster size (number of capitula in the cluster from which the individual capitulum was collected), "cap" is total number of capitula on the plant, "tertiary" is for a capitulum collected from a tertiary cluster, and "secondary" is for a capitulum collected from a secondary cluster. Similar response of *R. conicus* to plant and capitulum size was found on *C. nutans* (Sheppard *et al.*, 1994; but see McNeill & Fletcher, 2005).

In New Zealand, *R. conicus* adults emerge from overwintering around November (spring), when *C. tenuiflorus* and *C. pycnocephalus* are bolting, but three to four weeks before the first *C. nutans* capitula are formed (Jessep, 1989a). In those mid-spring weeks, *R. conicus* attack on the non-targets peaked at 35.7 ± 1.3% of capitula being attacked. Interestingly, even when *C. nutans* became available, oviposition on the non-targets did not cease completely, and 3.4 ± 0.8% of the capitula still were attacked. In contrast, 100% of early *C. nutans* capitula were attacked. *Carduus tenuiflorus* and *C. pycnocephalus* capitula are considerably smaller than *C. nutans* capitula (diameters 5.66 ± 0.018 mm, 7.23 ± 0.019 mm, and 19.73 ± 0.098 mm respectively), and they received up to 4 and 6 eggs per capitulum, respectively (with means of 0.17 ± 0.007 and 0.25 ± 0.009 for *C. tenuiflorus* and *C. pycnocephalus* respectively). In Southern California a biotype of *R. conicus* specialized on *C. pycnocephalus*

was reported to make up to 17 (mean 1.7) penetration holes per capitulum on that plant (Goeden & Ricker, 1985). Early *C. nutans* capitula are known to receive many dozens of eggs each (Jessep, 1989a; Woodburn, 1996a). In accordance with oviposition levels and capitulum size, *C. tenuiflorus* and *C. pycnocephalus* were usually found to support no more than one adult per capitulum (occasionally two, and rarely three adults per capitulum), which is comparable to the *C. pycnocephalus* specialized biotype (mean 0.9,Goeden & Ricker, 1985), whereas *C. nutans* capitula can support the successful development of dozens of adults (R. Groenteman, personal observations). Capitulum size appeared also to affect the size of adults, with *C. tenuiflorus* producing the smallest adults, followed by *C. pycnocephalus*, and *C. nutans* with the largest individuals (elytra length 3.13 ± 0.05 mm, 3.21 ± 0.04 mm, and 3.49 ± 0.02 mm respectively, $F_{2,1058} = 38.63$, $P < 0.0001$).

Although in this experiment the non-targets *C. tenuiflorus* and *C. pycnocephalus* did not appear to be attacked more in the presence of *C. nutans*, this was probably due to the spatial scale of the experiment which, considering the distance flown by the weevils to reach the site, was negligible. Therefore, the plots could not be truly considered far enough apart to create "*C. nutans*-present and -absent" treatments. Legal constraints regarding *C. nutans* propagation prevent any large scale manipulative experimentation on the species in New Zealand. *R. conicus* has not been considered a long distance disperser, mainly due to lack of knowledge (Sezen, 2007), and hence this was not considered a problem at the time the experiment was designed.

A field survey on a larger scale has revealed that non-targets are attacked by *R. conicus* more in the presence of *C. nutans* than in its absence (R. Groenteman, unpublished data). Furthermore, at times closer to the introduction of the weevil to New Zealand, *C. tenuiflorus* and *C. pycnocephalus* were commonly growing close to *C. nutans* (Jessep, 1989a). Currently, a population of *C. nutans* in sympatry with either *C. tenuiflorus* or *C. pycnocephalus* is hard to find (pers. obs.). It may be that the biological control agent has, in the many years since its introduction, successfully reduced populations of the non-targets where they were adjacent to *C. nutans*. The non-targets are still abundant in New Zealand; only, not so in proximity to *C. nutans*.

To conclude, although we were unable to show that the non-target species were attacked more in the presence of the target species, we have found that *R. conicus* effectively detects patches of the non-target species, and perhaps does not leave them quickly if *C. nutans* is in close proximity. Thus, the separation in time combined with the proximity in space is possibly improving biocontrol for all of these species: the non-targets are attacked by the less-likely-to-leave weevils early in the season, thus producing a second generation of *R.*

conicus. This second generation attacks *C. nutans* later in the season, after the first generation has already completed its life cycle, and when *C. nutans* is in its peak flowering in New Zealand.

Chapter 7: Changes in thistle abundance in Canterbury, New Zealand, 1985-2007 in relation to biocontrol

7.1 Introduction

There has long been criticism that control actions against weeds are based on insufficient objective impact and cost information (Cockayne, 1917; Hartley, 1983; Auld *et al.*, 1987; Bourdôt *et al.*, 2007). In many cases, even the information on weed occurrence and distribution is incomplete, and usually represents a snapshot, rather than trends of the weed's population dynamics (Bourdôt *et al.*, 2007). An additional criticism, often raised in weed biocontrol programmes, is of insufficient post-release monitoring (see refs within McFadyen, 1998). When monitoring takes place, it is usually to verify biocontrol agent establishment rather than weed population response to the agent (McClay, 1992). The following study on thistles in New Zealand offers a rare opportunity to assess quantitative changes in weed populations, in the context of a biocontrol programme, over a 20 year period.

Carduus nutans (nodding thistle) was first recorded in New Zealand in 1899 (Kirk, 1899). By 1940 it was already considered a noxious weed (Allan, 1940). The cost of *C. nutans* to the farming community was conservatively estimated as directly related to rosette size (i.e., rosette area equals land area of lost production) (Kelly & Popay, 1985), but it is now acknowledged that its impact on pasture growth is greater than rosette area (Thompson *et al*., 1987) and that it has adverse ecosystem-level effects, e.g., through. allelopaty (Wardle *et al.*, 1998). A *C.nutans* biological control programme in New Zealand was initiated in the early 1970s (Jessep, 1989a) and three insects were established.

In the mid 2000s *C.nutans* is still a noxious weed, and considered a problem in many regions of New Zealand (Roy *et al.*, 2004), including Canterbury (Environment Canterbury, 2006), although no work has been done to evaluate its current impact and cost, or the success of the biocontrol programme.

In the mid 1980s a weed population survey was conducted by G.W. Bourdôt and D. Kelly in several parts of New Zealand, including Canterbury, with the aim of quantifying the abundance of all the most common weeds of pasture. The survey identified thistles as the main weeds in the country's high producing pasture (Bourdôt & Kelly, 1986, only partial results published). The survey was powerful in that selection of participating paddocks was well randomized, and in that the method permitted relatively short times in each surveyed paddock, which enabled large areas to be covered. The strength of the survey lay also in the simplicity of the field method which assured decreased between-samplers error. The survey

estimated density and percent ground cover by the weeds some time after the introduction and establishment in 1972 of the first *C. nutans* biocontrol agent, the receptacle weevil *Rhinocyllus conicus*. Several years later further two biocontrol agents were introduced – nodding thistle crown weevil, *Trichosirocalus horridus sensu* (Panzer) (see Chapter 2 for clarification) in 1982 and nodding thistle gall fly, *Urophora solstitialis*, in 1992.

In the mid 2000s, an opportunity emarged to re-conduct the survey in part of the area surveyed in the mid 1980s. This was made possible owing to the availability of the original survey map-coordinate information and the detailed documentation of the process retained from the original 1980s survey. The aim was to revisit the same random sites and determine objectively whether the density of *C. nutans* had decreased over 20 years during which time two more biocontrol agents had been established. Anecdotal information from farmers indicated that *T. horridus* had been effective and they were finding *C. nutans* less of a management problem than in the past (Chapter 2). However, there were no quantitative data to test that proposition, hence this study.

The survey method, adopted from Batcheler (1971) was made suitable for simultaneous sampling of multiple species (Warren & Batcheler, 1979). This allowed us to study *C. nutans*, a (usually) biennial weed under a biocontrol programme, in relation to other thistle species: *Cirsium vulgare* (Scotch thistle) and *Cirsium arvense* (Californian thistle).

C. nutans and *C. vulgare* share similar life strategies – both are facultative-biennials, which rely on seeds for reproduction (Groves & Kaye, 1989; Popay & Medd, 1990). I therefore hypothesized that if any changes occurred to thistle populations from the mid 1980s to the mid 2000s, they should be of similar trends for *C. nutans* and *C. vulgare*, if it weren't for the biocontrol agents which target mainly *C. nutans*. Other factors aside biocontrol, like changes in weather, land use and management, may affect all thistle populations; yet, it was assumed that such factors would affect the two biennials *C. nutans* and *C. vulgare* similarly. Thus, the multiple thistles studied provide an internal control for evaluating biocontrol on *C. nutans*. Land management is arguably one of the most important factors affecting weed infestation in general and *C. nutans* infestation in particular (e.g., Dingwall, 1962; Bourdôt *et al.*, 2007). The main land management use change in Canterbury from the mid 1980s is the conversion of much high producing pasture from sheep farming to dairy farming.

7.2 Method

Five survey areas were selected in the 1980s for their proximity to where the surveying teams were based, in regions in both the North and South Islands of New Zealand. However, in the 2000s, the survey was only repeated in the South Island, in three regions in

Canterbury: Lincoln, Oxford and Ashburton. Each region covered one old series NZMS 1 topographic map (scale 1:63,360). The sampling unit was a paddock, and paddocks were selected using computer-generated random coordinates within the high producing pasture map units. The same paddocks were surveyed in the 1980s and in the 2000s. In Lincoln region, the sampling intensity was reduced between years in the 1980s: in 1984-85, 102 paddocks were sampled (one paddock per 1000 ha), of which only 62 (one paddock per 1666 ha) were revisited in 1985-86 and in the 2000s. This was done to reduce sampling effort, while maintaining reasonably small 95% confidence limits. In the 2000s, if paddocks were no longer in pasture, they were not surveyed. The survey was carried out in midsummer, when weed cover peaks. In the 1980s, six non-palatable herbaceous weeds were surveyed. Thistles, especially *C. nutans*, *C. vulgare* and *C. arvense*, emerged as the main contributors to weed cover in the South Island, and therefore in the 2000s thistles were the only weeds surveyed.

The method used in the field was the truncated point-distance nearest-neighbour technique (Batcheler, 1975). In each paddock 30 points at 7 m spacings along a randomly orientated sampling line were examined. The distance from each point to the nearest thistle occurring within a maximum search radius was measured. The search radius used in 1984-85 was 3 m. This was reduced to 1.25 m in 1985-86, as part of the reduced sampling effort. In the 2000s, a radius of 1.5 m was used. When a thistle was found, the distance from this plant to any neighbouring thistle (of any species) was measured, again with the same maximum search radius. The procedure was repeated once more to get the distance from the nearest neighbour to its nearest neighbour (Figure 1). The density of each species in each region (not in each paddock) was estimated from the distances from the points to individuals of that species, after correcting for clumping using the nearest and next-nearest neighbour distances (see Batcheler, 1971; and Warren & Batcheler, 1979 for details). The programme used to calculate the densities in the 1980s had to be recreated, based on Batcheler (1971; 1973; 1975), Warren & Batcheler (1979), and notes found with the original data from the 1980s. In addition, the original data were reanalyzed using the newly created program, to ensure that changes over time I discuss were not caused by differing methods of analysis. As a result of this reanalysis, the density estimates given here for the early surveys differ somewhat from those given in Bourdôt & Kelly (1986). Also note that some of the 1980s data are previously unpublished. The formulae for the density calculations are provided in Appendix C.

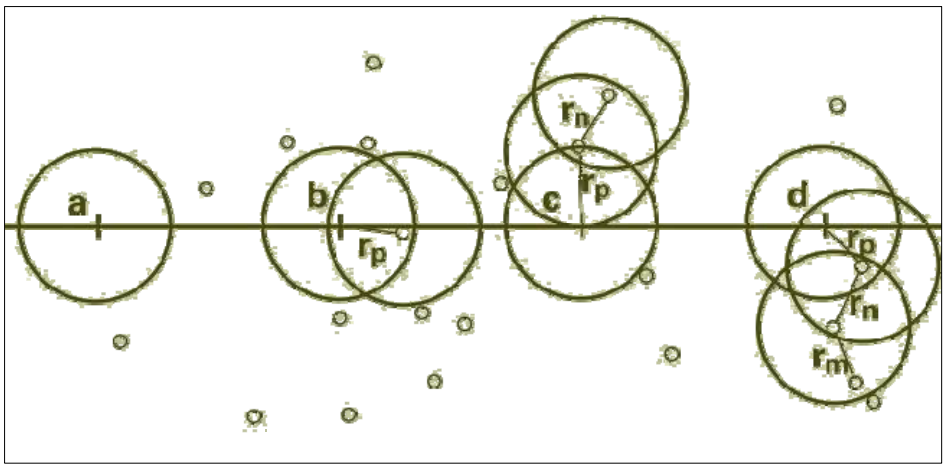

Figure 7.1: An illustration of the truncated point-distance nearest-neighbour technique, adapted from Batcheler (1975). Four sampling points (a-d) along one sampling transect are illustrated, each demonstrating one possible scenario: a. No thistle is encountered within the search radius; b. A thistle is encountered within the search radius. No neighbouring thistle is encountered within the same search radius from this thistle; c. A thistle is encountered within the search radius. A nearest thistle neighbour is encountered within the same search radius from this thistle, but no next nearest neighbour is encountered within the same search radius from the nearest neighbour; d. A thistle is encountered within the search radius. A nearest neighbour is encountered within the same search radius from this thistle, and a next nearest thistle neighbour is encountered within the same search radius from the nearest neighbour.

In 1984-85, each plant diameter was recorded, and later used to calculate plant area as the circle enclosed by this diameter. In all subsequent surveys, the length of the longest leaf of each plant was measured instead of diameter. In 1985-86, plant area was calculated as that of the circle enclosed by the longest leaf (Bourdôt & Kelly, 1986). However, in the 2000s a more accurate plant area estimates was used, based on the relationship between the longest leaf and actual measured rosette area, formulated by Kelly & Popay (1985) which, on average, generates values of about a third the area of the circle enclosed by the longest leaf. For large plants, the two methods would generate almost similar areas; the main difference is apparent in small rosettes, where more bare area is present between the leaves. The calculation of ground cover based on the area of the circle enclosed by the longest leaf is perhaps less accurate, but is still realistic since, although the area between thistle leaves may encompass good pasture, it is hardly grazed. Plant area was also recalculated for the mid 1980s data (with half the measured diameter regarded as the length of the longest leaf for 1984-85 season). Mean plant area was then combined with density to give an estimate of

percent ground cover. The presence of any thistle species in each paddock was also noted, regardless of their occurrence on the random sampling line.

Since a substantial conversion of high producing sheep and beef-stocked pasture to dairy farming has taken place between the 1980s and the 2000s, the densities, mean plant area, and percent ground cover were also separately calculated for dairy versus non-dairy farms.

Of the three regions surveyed, only Lincoln was visited twice in the mid 1980s (the only region of the three for which results have been published). With the high year-to-year variation in thistle density, this posed a genuine difficulty in interpreting the differences between a single year in the 1980s and multiple years in the 2000s for Oxford and Ashburton. Yet, I was able to set the single year in context for Ashburton, by using high resolution data that were collected by D. Kelly on a small scale, between 1985 and 1988, for *C. nutans*, *C. arvense* and *Carduus tenuiflorus* (winged thistle) at Northbank farm near Rakaia (located in the North East part of the Ashburton area in my survey). These data were collected as part of a thistle population demography study, in an irrigated site (1985-1988) and in a non-irrigated site (1986-1988) using a different method (for details of the method refer to Shea & Kelly, 1998). Only data collected in summer times, similar to the current survey time of year, were used. The possibility to put 1985-86 in context for Ashburton is extremely valuable, since Ashburton emerged in my survey as the area with the highest *C. nutans* densities.

Carduus nutans biocontrol agents were recorded in the 2000s where they were encountered, either on any of the thistle species along the sampling line or elsewhere in the paddock. A frequency was calculated for the occurrence (present/absent at the paddock level) of each of the three species of biocontrol agents in only those paddocks that had *C. nutans* present; this was then compared, using Chi-square tests, to the frequency of the biocontrol agents presence in paddocks only hosting thistles other than *C. nutans* (not empty of thistles altogether). The occurrence of biocontrol agents in paddocks with and without *C. nutans* (but not empty of thistles) was also analyzed in a generalized linear model ('glm' command in R) (R Development Core Team, 2006) with *C. nutans* presence, region and year as main effects. Biocontrol agent occurrence was compared also between dairy farms vs. other farms types using Chi-square tests.

7.3 Results

All the paddocks visited in the 1980s surveys were successfully relocated in the 2000s, and it was possible to resurvey most of them. The number of paddocks visited is given in Table 7.1. Thistles, which were identified as the main weeds in high producing pastures in the

mid 1980s survey (Bourdôt & Kelly, 1986), were confirmed to remain significant weeds in the mid 2000s.

Thable 7.1: The number of paddocks surveyed in each region and year

		Number of paddocks surveyed in:		
Surveyed by:	Year	Lincoln	Oxford	Ashburton
Dave Kelly and Graeme Bourdôt	1984-85	102	Not surveyed	Not surveyed
	1985-86	62	51	64
Ronny Groenteman and assistants	2004-05	Not surveyed	Not surveyed	57
	2005-06	59	46	63
	2006-07	56	47	59

Regions varied in the most important thistle – no one species emerged as most important in all three regions in either density, size, or contribution to percent ground cover (Table 7.2).

Total thistle densities did not differ markedly between regions, but each region was dominated by a different species, and the balance between species within each region has somewhat shifted since the mid 1980s (Table 7.2; Figure 7.2). *Carduus nutans*, which appeared important only in Ashburton region in the mid 1980s, remained important there, and remained relatively unimportant in Lincoln and Oxford. *Cirsium vulgare* density has decreased in all three regions (although it is impossible to determine whether the population decrease is significant in the current versus the early survey). *Cirsium arvense* density has risen in both Lincoln and Ashburton (Table 7.2).

C.nutans mean plant area has decreased in Ashburton and Lincoln, but increased in Oxford. *Cirsium vulgare* mean area increased in all three regions, and *C. arvense* mean area remained more or less unchanged (Table 7.2; see also leaf lengths in Figures 7.2 & 7.3).

Total percent ground cover by thistles decreased in all three regions, but different species contributed differently to that total result: *C. nutans* cover decreased in Lincoln and Ashburton and increased in Oxford; *C. vulgare* cover decreased in all three regions; and *C. arvense* cover either increased in cover or remained unchanged (Table 7.2).

Table 7.2: (**a**) Density (plants per hectare), (**b**) mean plant area (m^2), and (**c**) % ground cover by thistles in three regions of Canterbury, in the mid 1980s and in 2005-2007.

	Lincoln					**Oxford**				**Ashburton**				
	1984-85	1985-86	2005-06	2006-07	Ratio[1]	1985-86	2005-06	2006-07	Ratio[2]	1985-86	2004-05	2005-06	2006-07	Ratio[2]
(a) Density[3]														
C. nutans	53	106	40	52	0.58	23	81	16	2.11	1830	1820	272	1024	0.57
C. vulgare	221	1095	62	182	0.19	1310	378	678	0.40	211	33	22	43	0.15
C. arvense	189	223	361	358	1.75	1492	1579	1087	0.89	244	649	600	1072	3.17
C. tenuiflorus				3										
total density	463	1425	459	595	0.56	2825	2038	1789	0.68	2283	2502	894	2139	0.81
(b) Plant area[4]														
C. nutans	0.0282	0.0738	0.0253	0.0259	0.50	0.0139	0.0209	0.0963	4.20	0.0330	0.0178	0.0247	0.0180	0.61
C. vulgare	0.0192	0.0192	0.0300	0.0292	1.54	0.0217	0.0402	0.0221	1.43	0.0243	0.0255	0.0481	0.0248	1.35
C. arvense	0.0038	0.0136	0.0109	0.0097	1.19	0.0100	0.0114	0.0082	0.98	0.0129	0.0113	0.0091	0.0116	0.83
C. tenuiflorus				0.0130										
total area	0.0174	0.0225	0.018	0.0197	0.95	0.0174	0.0221	0.0166	1.11	0.0305	0.0164	0.0164	0.0154	0.53
(c) % ground cover														
C. nutans	0.0187	0.0786	0.0123	0.0155	0.29	0.0036	0.0218	0.0180	5.50	0.6124	0.3279	0.0741	0.1905	0.32
C. vulgare	0.0531	0.2113	0.0225	0.0614	0.32	0.3205	0.1957	0.1752	0.58	0.0520	0.0085	0.0117	0.0110	0.20
C. arvense	0.0089	0.0304	0.0478	0.0400	2.23	0.1687	0.2324	0.1043	1.00	0.0319	0.0743	0.0605	0.1287	2.75
C. tenuiflorus				0.0004										
total cover	0.0807	0.3203	0.0826	0.1174	0.50	0.4928	0.4499	0.2975	0.76	0.6964	0.4107	0.1462	0.3302	0.42

[1]2000s mean divided by 1980s mean
[2]2000s mean divided by 1985-86 value
[3]Plants per hectare
[4]Rosette area in m^2

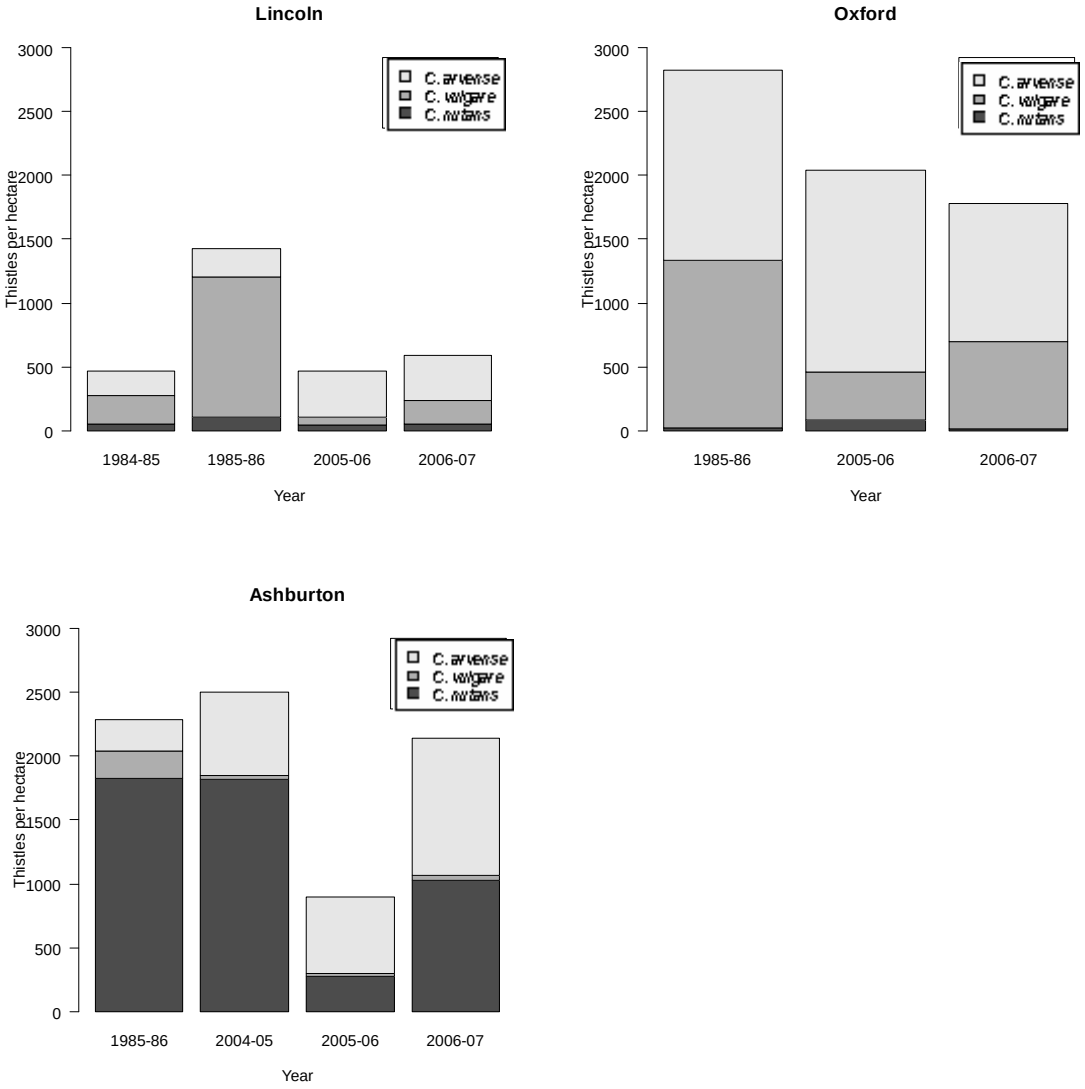

Figure 7.2: Thistle densities in three regions of Canterbury in the 1980s and in the 2000s

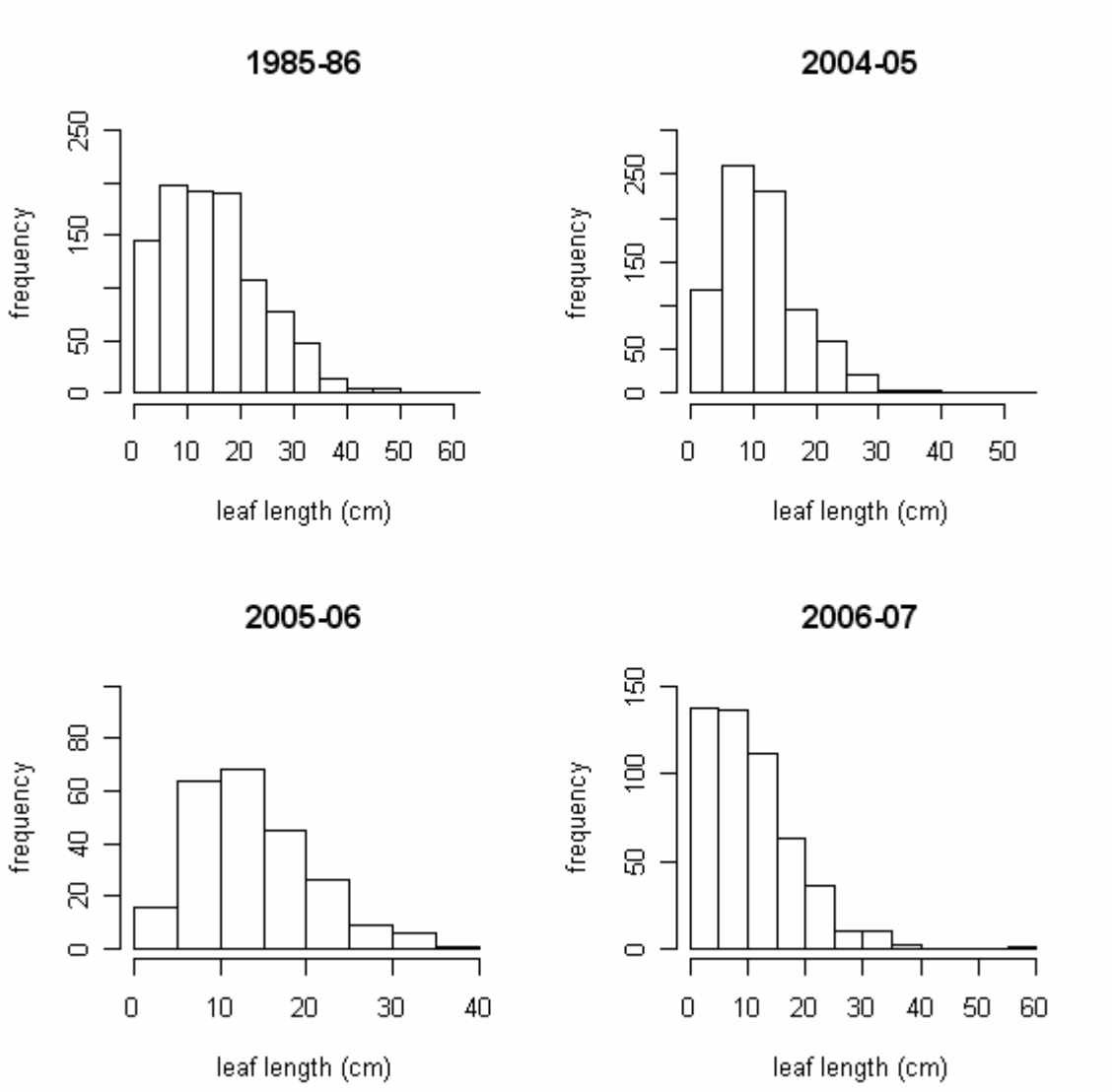

Figure 7.3 *C. nutans* longest leaf lengths distribution in Ashburton.

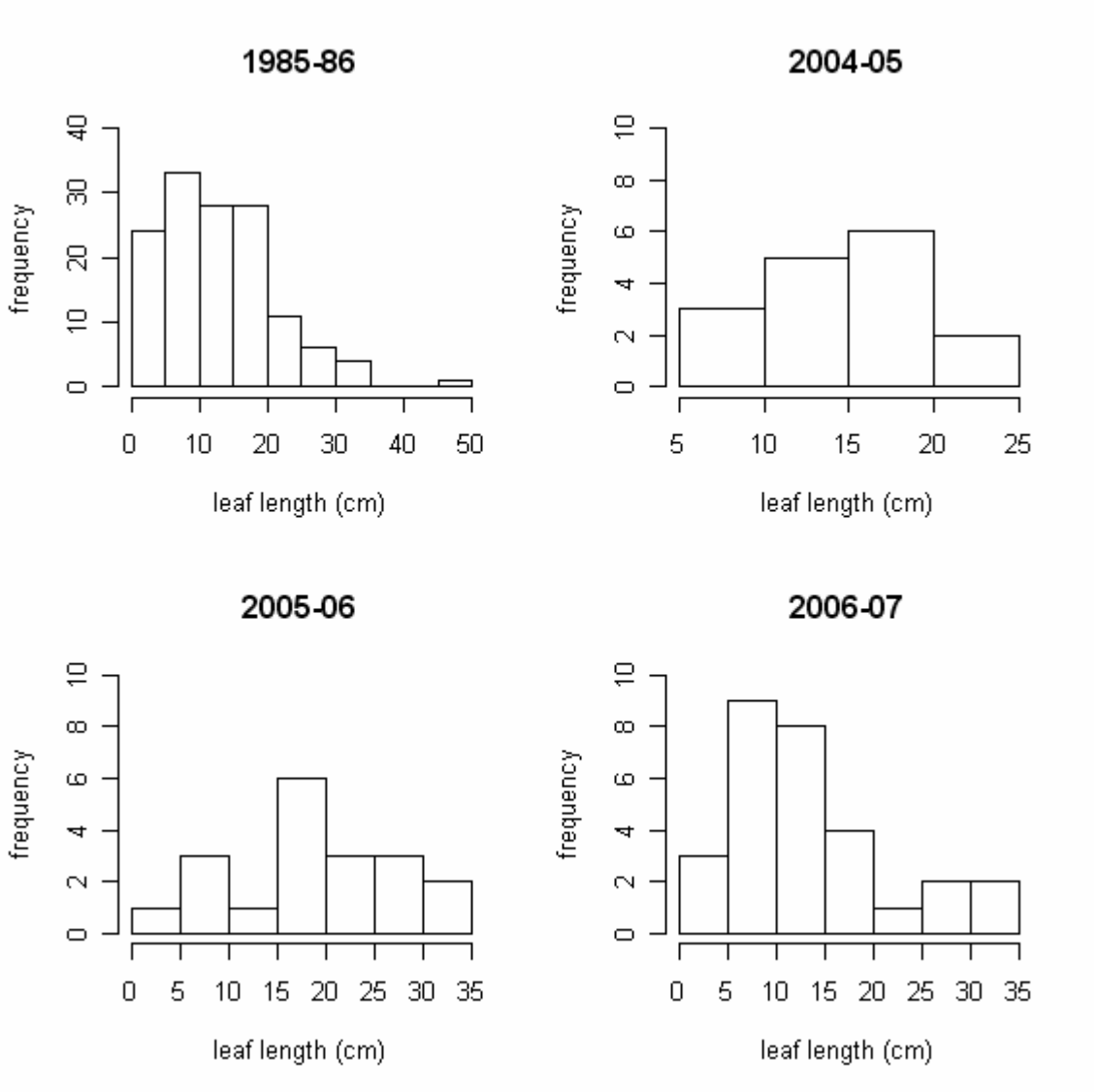

Figure 7.4 *C.vulgare* longest leaf lengths distribution in Ashburton.

In the Lincoln region, 1985-86 emerges as a year of high thistle density compared to 1984-85; and from the high resolution data presented in Table 7.3 it is concluded that in Ashburton, too, 1985-86 was a year of high thistle density compared to the two following years. The values presented in Table 7.3 represent a very small sampling area located specifically because it contained *C. nutans* and are therefore not comparable to the per-hectare values calculated from the current survey.

Table 7.3: (**a**) Density (plants per hectare), (**b**) mean plant area (m^2), and (**c**) % ground cover by thistles in an irrigated and a non-irrigated site in Rakaia (near Ashburton) in the mid to late 1980s.

	Irrigated				**Non-irrigated**		
	1985-86	1986-87	1987-88	Ratio[1]	1986-87	1987-88	Ratio[2]
(a) Density[3]							
C. nutans	229667	9667	27333	12.41	54000	92000	3.15
C. vulgare	11000	2333	1000	6.60	333	333	33.00
C. tenuiflorus	1333		1000	2.67			
Total	242000	12000	29333	11.71	54333	92333	3.30
(b) Plant area[4]							
C. nutans	0.0180	0.0429	0.0023	0.79	0.0013	0.0014	13.22
C. vulgare	0.0048	0.0002	0.0008	9.62	0.0001	0	159.46
C. tenuiflorus	0.0001		0.0023	0.06			
Total	0.0180	0.0337	0.0022	1.00	0.0013	0.0014	13.23
(c) % ground cover							
C. nutans	23.31	5.52	0.64	7.57	0.77	1.32	22.37
C. vulgare	0.29	0.01	0.01	29.95	0	0	1452.61
C. tenuiflorus	0		0.03	0.13			
Total	23.60	5.53	0.68	7.60	0.77	1.32	22.64

[1]1985-86 divided by the mean of 1986-87 and 1987-88 on the same (irrigated) site
[2]1985-86 divided by the mean of 1986-87 and 1987-88 on the adjacent (non-irrigated) site
[3]Plants per hectare
[4]Rosette area in m^2

A comparison of thistles in dairy paddocks and other farm types in the 2000s reveals no pattern that fits all three regions (Table 7.4). In Ashburton, where the conversion to dairy farming was highest, thistle densities and ground cover were much lower in dairy farms than in the other farm types. In years of high density (2004-05 and 2006-07), *C. nutans* plants in dairy farms were larger on average than those in other farm types. In Lincoln, thistle densities and ground cover were higher in dairy farms than in other farm types (mainly due to *C. arvense*), and in Oxford there was high variation between years and farm types.

Table 7.4: (**a**) Density (plants per hectare), (**b**) mean plant area (m^2), and (**c**) % ground cover by thistles in dairy vs. in other management types paddocks in three regions (% of paddocks converted) of Canterbury, in 2005-2007.

	Lincoln (10)				**Oxford (11)**				**Ashburton (28)**					
	2005-06		2006-07		2005-06		2006-07		2004-05		2005-06		2006-07	
	dairy	other	dairy	other	dairy	other	dairy	other	dairy	other	dairy	other	dairy	other
(a) Density[1]														
C. nutans	0	43	106	49	0	90	0	16	75	2853	64	380	205	1519
C. vulgare	0	67	761	132	217	406	94	770	0	49	46	8	0	54
C. arvense	1101	259	987	299	1936	1565	61	1252	60	975	11	927	82	1638
C. tenuiflorus			0	3	1	0								
total	1101	369	1854	483	2153	2061	155	2038	135	3877	121	1315	287	3211
(b) Plant area[2]														
C. nutans	0	0.0253	0.0423	0.0242	0	0.0209	0.0794	0.0992	0.0202	0.0177	0.0293	0.0240	0.0398	0.0143
C. vulgare	0	0.0300	0.0382	0.0259	0.0426	0.0401	0.0636	0.0211	0.0059	0.0268	0.0562	0.0369	0.0203	0.0260
C. arvense	0.0117	0.0104	0.0119	0.0091	0.0199	0.0105	0.0280	0.0080	0.0044	0.0118	0.0080	0.0091	0.0115	0.0116
C. tenuiflorus			0	0.0130	0	0								
total	0.0117	0.0199	0.0264	0.0179	0.0230	0.0220	0.0552	0.0159	0.0153	0.0165	0.0335	0.0150	0.0334	0.0134
(c) % ground cover														
C. nutans	0	0.0138	0.0485	0.0140	0	0.0246	0	0.0184	0.0175	0.5098	0.0167	0.1005	0.0861	0.2210
C. vulgare	0	0.0255	0.3141	0.0402	0.0957	0.2135	0.0665	0.1889	0	0.0132	0.0231	0.0032	0	0.0143
C. arvense	0.1293	0.0342	0.1267	0.0319	0.3994	0.2156	0.0190	0.1162	0.0031	0.1157	0.0008	0.0933	0.0099	0.1941
C. tenuiflorus			0	0.0005	0	0								
total	0.1293	0.0734	0.4893	0.0866	0.4951	0.4536	0.0855	0.3235	0.0206	0.6387	0.0405	0.1971	0.0960	0.4295

[1]Plants per hectare
[2]Rosette area in m^2

Carduus nutans biocontrol agents, which were recorded in the 2000s surveys, are known to prefer *C. nutans*, but are also known to attack other thistle species. Although they were almost always found more in paddocks hosting *C. nutans* than in paddocks hosting only other thistle species, the difference was non significant in most cases in the Chi-squared tests (Table 7.5); however, *C. nutans* presence was a significant predictor of biocontrol agents presence in the generalized linear model (Table 7.6). It is interesting to note that *U. solstitialis*, which is more restricted to *Carduus* spp., was recorded once in a paddock not hosting *C. nutans* (Table 7.5). This specific paddock only hosted *C. arvense*, and it is possible that the fly encountered was in fact a misidentified *Urophora cardui* (Californian thistle gall fly) which is present albeit at low density in the area.

Table 7.5: Percentage of paddocks hosting *C. nutans* and paddocks not hosting *C. nutans* but hosting other thistles (not thistle-free) that had (**a**) *R. conicus*, (**b**) *U. solstitialis*, and (**c**) *T. horridus* encountered in.

	% paddocks **with** *C. nutans* (n) in which:	% paddocks **without** *C. nutans* but with other thistles (n) in which:	χ^2	***P***
(a) *R. conicus* was encountered				
Oxford 2005-06	10.0 (10)	0 (35)	3.25	0.07
Oxford 2006-07	16.7 (12)	18.2 (33)	0.01	0.92
Lincoln 2005-06	25.0 (20)	0 (26)	5.77	0.02
Lincoln 2006-07	41.7 (24)	13.3 (30)	3.24	0.07
Ashburton 2004-05	22.2 (45)	0 (10)	2.15	0.14
Ashburton 2005-06	32.6 (43)	0 (6)	1.89	0.17
Ashburton 2006-07	34.1 (44)	23.1 (13)	0.31	0.58
mean	26.0	7.8		
(b) *U. solstitialis* was encountered				
Oxford 2005-06	10.0 (10)	0 (35)	3.25	0.07
Oxford 2006-07	16.7 (12)	0 (33)	4.92	0.03
Lincoln 2005-06	5.0 (20)	0 (26)	1.27	0.26
Lincoln 2006-07	33.3 (24)	3.3 (30)	6.10	0.01
Ashburton 2004-05	35.6 (45)	0 (10)	3.39	0.07
Ashburton 2005-06	23.3 (43)	0 (6)	1.36	0.24
Ashburton 2006-07	25.0 (44)	0 (13)	3.10	0.09
mean	21.3	0.5		
(c) *T. horridus* was encountered				
Oxford 2005-06	0 (10)	0 (35)	0	1
Oxford 2006-07	8.3 (12)	0 (33)	2.59	0.11
Lincoln 2005-06	0 (20)	0 (26)	0	1
Lincoln 2006-07	16.7 (24)	6.7 (30)	1.07	0.30
Ashburton 2004-05	0 (45)	0 (10)	0	1
Ashburton 2005-06	0 (43)	0 (6)	0	1
Ashburton 2006-07	22.7 (44)	0 (13)	2.83	0.09
mean	6.8	1.0		

Table 7.6: ANOVA table for the effects of *C. nutans* presence, survey region and year on the proportion (logit transformed) of paddocks in which (**a**) *R. conicus*, (**b**) *U. solstitialis* and (**c**) *T. horridus* were encountered

(a) *R. conicus*

	df	Deviance	Resid. df	Resid. Dev.	*P*
NULL	350	350.73			
C. nutans present	1	24.57	349	326.16	<0.001
Region	2	1.53	347	324.63	0.470
Year	1	8.55	346	316.08	0.003

(b) *U. solstitialis*

	df	Deviance	Resid. df	Resid. Dev.	*P*
NULL	350	287.389			
C. nutans present	1	54.313	349	233.076	<0.001
Region	2	3.181	347	229.896	0.204
Year	1	0.059	346	229.837	0.808

(c) *T. horridus*

	df	Deviance	Resid. df	Resid. Dev.	*P*
NULL	348	135.901			
C. nutans present	1	8.655	347	127.246	<0.001
Region	2	2.198	345	125.048	0.062
Year	1	32.045	344	93.003	<0.001

For the most part, biocontrol agent presence in dairy farms was not significantly different from their presence in other farm types (Table 7.7). There were only two significant differences, both involving *U. solstitialis*; in one of them, fly presence was greater in dairy farms, and in the other it was greater in other farm types (Table 7.7).

Table 7.7: Percentage of dairy paddocks and non-dairy paddocks that had (**a**) *R. conicus*, (**b**) *U. solstitialis*, and (**c**) *T. horridus* encountered in.

	% **dairy** paddocks (n) in which:	% **other (non-dairy)** paddocks (n) in which:	χ^2	***P***
(a) *R. conicus* was encountered				
Oxford 2005-06	0 (5)	2.4 (41)	0.12	0.73
Oxford 2006-07	0 (5)	19.1 (42)	0.94	0.33
Lincoln 2005-06	0 (6)	9.4 (53)	0.56	0.45
Lincoln 2006-07	16.7 (6)	26.0 (50)	0.16	0.69
Ashburton 2004-05	35.3 (17)	17.5 (40)	1.28	0.26
Ashburton 2005-06	11.8 (17)	26.1 (46)	0.99	0.32
Ashburton 2006-07	18.8 (16)	34.9 (43)	0.81	0.37
mean	11.8	19.3		
(b) *U. solstitialis* was encountered				
Oxford 2005-06	0 (5)	2.4 (41)	0.12	0.74
Oxford 2006-07	0 (5)	4.8 (42)	0.24	0.63
Lincoln 2005-06	16.7 (6)	0 (53)	7.70	0.01
Lincoln 2006-07	16.7 (6)	16.0 (50)	0	0.97
Ashburton 2004-05	0 (17)	25.0 (40)	4.00	0.05
Ashburton 2005-06	17.7 (17)	15.2 (46)	0.04	0.84
Ashburton 2006-07	18.8 (16)	18.6 (43)	0	0.99
mean	10.0	11.7		
(c) *T. horridus* was encountered				
Oxford 2005-06	0 (5)	0 (41)	0	1
Oxford 2006-07	0 (5)	2.4 (42)	0.12	0.73
Lincoln 2005-06	0 (6)	0 (53)	0	1
Lincoln 2006-07	0 (6)	12.0 (50)	0.71	0.40
Ashburton 2004-05	0 (17)	0 (40)	0	1
Ashburton 2005-06	0 (17)	0 (46)	0	1
Ashburton 2006-07	25.0 (16)	13.9 (43)	0.69	0.41
mean	3.6	4.0		

7.4 Discussion

7.4.1 Thistle life strategies effect on year-to-year variation

C. nutans is a monocarpic facultative-biennial herb which relies solely on seeds for reproduction (Popay & Medd, 1990). Plant age and exposure to low temperatures determine if a plant will flower in a certain summer or remain at the vegetative rosette stage for another year (Medd, 1977; Popay & Medd, 1990). Thus, population size distribution in early summer generates information on its age structure: small plants are most likely current year's recruitment, whilst large plants are most probably previous year's survivors that will flower in the current summer (Popay *et al.*, 1979). Bare ground, as often found at the end of summer in heavily-grazed pasture, presents conditions favourable for *C. nutans* recruitment from its long lived seed bank (Phung & Popay, 1981); a rainy summer that enhances pasture growth would

reduce recruitment. This creates strong year-to-year variation in *C. nutans* density. This is well demonstrated by the difference (within a single year) in the adjacent irrigated and non-irrigated sites in Rakaia (Table 7.3; e.g., either 1986-87 or 1987-88). Precipitation and grazing regimes are probably responsible for year-to-year variation in my survey density results both in the 1980s and in the 2000s.

Cirsium vulgare too behaves as a biennial in New Zealand (Hartley, 1983), although its germination is less affected by pasture cover than *C. nutans* germination (Phung & Popay, 1981). In contrast, *C. arvense* is a perennial that reproduces vegetatively, and although pasture cover affects its germination as much as it affects *C. nutans* (Phung & Popay, 1981), this has little effect on *C. arvense* populations over short time periods; thus it is not surprising that *C. arvense* density did not exhibit high yearly variation in the surveys. Pasture cover does however affect *C. arvense* shoot density (G.W. Bourdôt, pers. comm.), which is likely to affect populations over longer time scales.

7.4.2 Densities and size in relation to life strategy

C. nutans densities in Ashburton in the mid 1980s and in the mid 2000s were fairly similar (Table 7.2), suggesting that recruitment has not changed much. It is therefore unlikely that the introduced seed predators, *R. conicus* and *U. solstitialis*, affected seed production sufficiently to deplete *C. nutans* seed bank to the point that *C. nutans* is seed limited (Shea & Kelly, 1998; Shea *et al.*, 2005; Chapter 3). Unlike *C. nutans*, *C. vulgare* appears to have dramatically decreased in density in Ashburton region.

In mean plant area I have, however, recorded a decrease for *C. nutans*, which could imply changes to the population age structure. This is further reinforced by the size distribution, as illustrated in Figure 7.3: in 1985-86, despite this being a year of high recruitment, large plants (longest leaf length of 10 cm or longer; probably survivors from the previous year), comprised a high proportion of the population (high survival rate); whereas in the mid 2000s, a large proportion of the population had longest leaf length of 10 cm or shorter, mainly in high recruitment years (2004-05 and 2006-07) but also in the low recruitment year (2005-06). In comparison, *C. vulgare*, which, despite great difference in absolute density, showed a similar size distribution to *C. nutans* in 1985-86, was skewed towards larger plants in the mid 2000s (Figure 7.4). Thus, while *C. vulgare* apparently showed low recruitment and high survival compared to the mid 1980s, *C. nutans* apparently showed high recruitment, not different to the 1980s, but lower survival.

Interestingly, a similar relative decrease in percent ground cover was generated by *C. nutans* and *C. vulgare* in Ashburton and Lincoln (2000s to 1980s ratios, Table 7.2). But while

in *C. vulgare* the decrease in cover appears to have been driven by a decrease in density, in *C. nutans*, the decrease in mean plant area was apparently the driver.

I have no explanation for the difference in the trends of density change between the two biennial thistles. However, the decrease in *C. nutans* size may be related to the activity of the biocontrol agent *T. horridus* (nodding thistle crown weevil). The weevil decreases *C. nutans* rosettes survival (Woodburn, 1997; Chapter 4), hence fewer rosettes reach their second year. It also reduces the growth of surviving rosettes (Woodburn, 1997; Chapter 4). These two effects have probably driven the change in *C. nutans* population age structure, evident as a decrease in mean plant area (Table 7.2).

Cirsium arvense appears to have sustained a stable size not only throughout the years, but also throughout the three regions (Table 7.2). This is not surprising, because *C. arvense* is perennial which does not rely on seeds for reproduction. Its population age structure (and therefore size) is therefore less affected by factors which could reduce germination (be it the biocontrol agents or environmental conditions). In addition, *C. arvense* is not attacked by *T. horridus*, the agent that is associated with reduced rosette size.

7.4.3 Densities and size in relation to farm management

For many years, livestock farming in Canterbury consisted largely of sheep farming, with dairy only comprising around 1% by livestock numbers (Dingwall, 1962). The mid 1980s survey points were randomly selected from areas of high producing pasture, which was carrying mainly sheep and some beef cattle. In recent years, Canterbury has seen a trend of conversion from sheep to dairy farming - two farm types greatly differing in management.

Pasture management has long been acknowledged as an important tool in keeping thistle populations controlled (Dingwall, 1962; Phung & Popay, 1981; Edmonds & Popay, 1983; Martin & Rahman, 1988a; Bourdôt *et al.*, 2007); but it has also been long recognized that this is not an easy task to achieve in the climatic conditions, the soil and landscape of Canterbury (Dingwall, 1962), and is still acknowledged as difficult in the 21st century (Bourdôt *et al.*, 2007). Dairy farm management, however, presents a different case altogether: the pasture is regularly irrigated, fertilized, and not as intense grazed as in other farm types. The main effect these farm practices have on thistles is by producing strong pasture cover, which inhibits thistle germination (Phung & Popay, 1981; Popay & Medd, 1990). Strong pasture also competes better for water and nutrients, which reduces thistle seedling survival (Popay & Kelly, 1986; Popay & Medd, 1990). Dense pasture can easily smother young thistle rosettes to death (Phung & Popay, 1981; Edmonds & Popay, 1983). Pasture management is insufficient, though, to control large, established thistle rosettes (Martin & Rahman, 1988b).

In addition, weed management, especially chemical, is more intense in dairy farming (Holland & Rahman, 1999), as part of the overall more intensive management. Yet, I found no pattern of thistle density that could be attributed to farm type management that fits all three (Table 7.4). Only in Ashburton can I say that dairy farms had fewer thistles than 121 farm types, as would be expected. Perhaps the larger relative conversion to dairy in Ashburton (28% of surveyed paddocks) made this trend clearly apparent.

I would have expected the two biennial species to be affected similarly by land management changes. Yet Table 7.4 shows that *C. nutans* was reduced more than *C. vulgare* on dairy farms. This could not be clearly attributed to the biocontrol agents, since their occurrence was not different on dairy and on other farm types (Table 7.7). It therefore appears that *C. nutans* may be tolerated less than *C. vulgare* on dairy farms and may be more actively managed. *Carduus nutans* well warrants this discrimination as, unlike other thistles (and other broad-leaved weeds in New Zealand pastures), it inhibits clovers and induces long-term decline of soil nitrogen (Wardle *et al.*, 1998); yet this is expected to occur in all farm types.

No pattern was revealed that can explain differences in mean plant area between farm types in the three regions. Interestingly, in Ashburton, in years of high recruitment (2004-05 and 2006-07), *C. nutans* mean area on dairy farms was larger than on other farm types. This implies that the populations on the dairy farms are dominated by survivors and not by new recruitment. In general, it is plausible that differences in percent ground cover between management types were driven by density rather than plant size.

7.4.4 Success of biocontrol on *C. nutans*

Some evidence indicates that farmers are generally satisfied with *C. nutans* status at present. This is true for Hawkes Bay in the North Island of New Zealand, (I. Popay, pers. comm.), where *C. nutans* has been considered a major weed, and for the South Island (Chapter 2). However, I have recorded no major changes to *C. nutans* density around Ashburton or across all three areas in Canterbury. Firstly the densities in Ashburton in the 2000s only once were as high as they had been in 1985-86 and the mean density in the 2000s was only 57% of the earlier number, but the evidence in Tables 7.1 and 7.2 suggests that 1985-86 was a relatively high year for the 1980s, so the apparent decrease is probably largely due to weather rather than biocontrol. Secondly, *C. nutans* actually increased around Oxford, albeit that it has always been rare in this area so the changes may be more affected by stochasticity. Thirdly, I hypothesised that because of biocontrol *C. nutans* would decrease more than *C. vulgare*, but the opposite was true in all three areas. Overall there is little support from this 20-year

random-site study for the idea that *C. nutans* biocontrol has effectively reduced the density of this pasture weed, despite reports of farmers being pleased.

It is possible that the lack of change in fact masks genuine changes. In the following concluding remarks I suggest mechanisms that may account for the satisfaction expressed by farmers despite no observed decrease in *C. nutans* density.

First, I compare *C. nutans* control strategies in the past and at present times. In the early 1950s herbicides were already widely in use in New Zealand for *C. nutans* control (Delahunty, 1960). The main herbicides in use were the synthetic auxins MCPA, MCPB, 2,4-D and 2,4-DB. The former two are effective against thistles, but are harmful to broad-leaved pasture plants (clovers, lucerne) (e.g., Delahunty, 1960; Delahunty, 1962); while the latter two, though less damaging to the pasture, are less effective in thistle control (Delahunty, 1960; Bourdôt *et al.*, 2007). Complete control by herbicides was realized to be unachievable early on (Delahunty, 1962), and thistle resistance to herbicides quickly evolved (Harrington *et al.*, 1988; Bonner *et al.*, 1998). Despite their reduced efficiency, herbicides remained the main method for control for long, since they offered the most cost-effective solution.

The commencement of the biological control programme against *C. nutans* in the early 1970s is likely to have allowed some decrease in herbicide use (Bourdôt *et al.*, 2007, p. 149). This might have been further enhanced by the removal of government subsidies on herbicides, starting from the mid 1980s (Evans *et al.*, 1996) (but see Holland & Rahman, 1999). This may have allowed herbicide-susceptible pastures to grow stronger, which would have decreased *C. nutans* recruitment and out-competed thistle seedlings. Thus, the biocontrol programme may have indirectly contributed to maintenance of *C. nutans* densities at a stable level (albeit weedy still), with substantially decreased investment from the farmers (Figure 7.5). This could well explain farmer satisfaction with the current, unchanged, *C. nutans* status.

Another possible explanation for farmer satisfaction may lie more directly with the biocontrol programme, and more specifically, with the crown feeding weevil agent *T. horridus*. As previously discussed (e.g., Section 4.2), this agent may account for the recorded decrease in *C. nutans* mean plant size, which implies that fewer plants survive to reach the flowering stage. Thus, flowering stands may now be less conspicuous at a given rosette density per hectare compared to past infestations, which would justify farmer satisfaction.

Finally, I consider the possible combined effect of the three biocontrol agents on restricting *C. nutans* from further spreading in New Zealand. It is accepted that in the absence of control, as well as in the presence of either seed predators, *C. nutans* populations in New Zealand are expected to be continuously growing (Shea & Kelly, 1998; Shea *et al.*, 2005). Yet the three agents together are quite possibly having a strong enough effect that at least

stabilizes *C. nutans* populations, and prevents them from further spreading. In addition, with the increasing awareness and legislative effort against weed spreading by means of human activity, it is likely that farmers are more vigilant in preventing further spreading of *C. nutans*.

ombined effects of farmer vigilance and biocontrol agent activity is another proposed mechanism that could explain the current contained status of *C. nutans*, which maintains satisfaction amongst farmers.

Thus, despite the lack of a demonstrated major difference in *C. nutans* population density between the mid 1980s and the mid 2000s, my results are consistent with the idea that the impact of biological control has not been to decrease the density of this weed, but rather to decrease the cost of management input required to maintain the current densities. Clearly to prove this hypothesis will require sampling not just thistle densities, but also management inputs, which is beyond the scope of the present study, but well worthwhile.

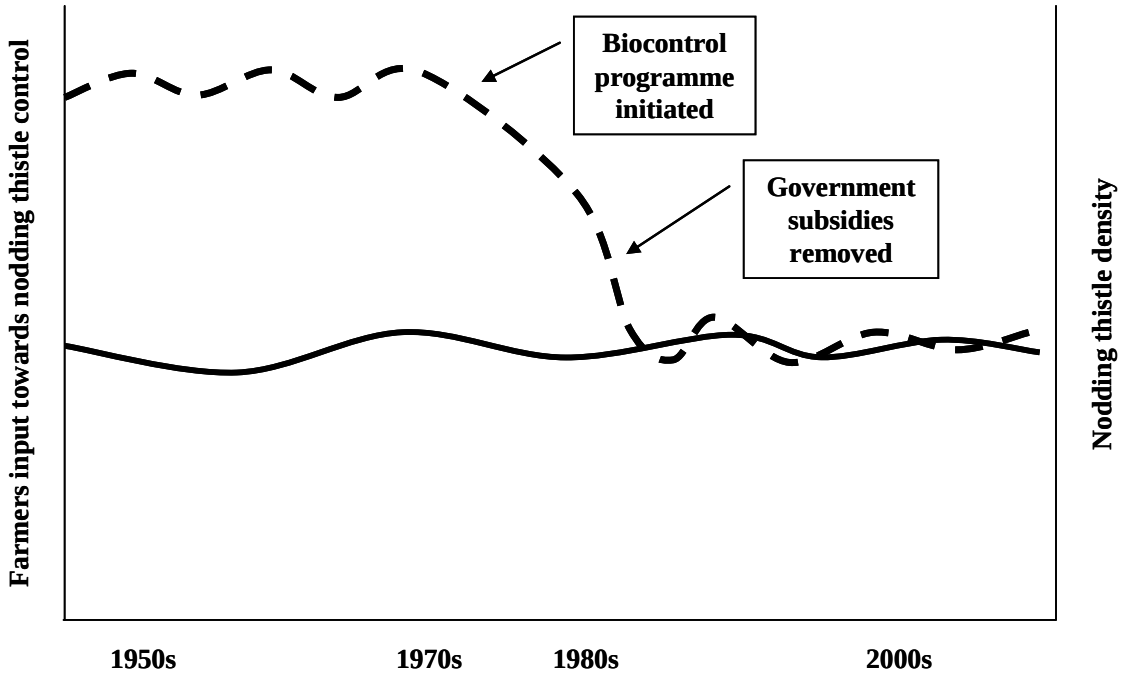

Figure 7.5: An illustrated proposed mechanism for (no) changes in *C. nutans* density (solid line, observed density in the field) in relation to input by farmers towards its control (dashed line, predicted density in the absence of active management by farmers). It is suggested that farmers input decreased slightly following the initiation of the biocontrol programme in 1972, and decreased further following the removal of government subsidies on herbicides in the late 1980s.

Chapter 8: Synthesis

Classical biocontrol systems involve multiple species and are therefore complex and challenging. In some respects the complexity is reduced by the exclusion of some trophic levels (e.g., parasitoids of the biocontrol agent or some competing herbivores), which exist in the native range, from entering the introduced range. Nonetheless, when biocontrol agents are introduced into a new environment, they are likely to encounter both new hosts and new enemies. They form new links in pre-existing food-webs. Biocontrol practitioners are faced with the challenges involved in trying to predict various aspects of the outcome of this community assembly exercise, including, most importantly, the effectiveness of weed suppression. In this thesis I used thistle biocontrol in New Zealand to explore how aspects of a multiple species system affect aspects of biocontrol effectiveness; in this final chapter, I place these aspects in a broader context.

8.1 Population models - a tool for improving effectiveness in weed biocontrol

An ineffective biocontrol agent does not only fail to suppress its target weed; it also often remains in the system, sometimes at high abundance (since its host remains abundant), and is available to native predators and parasitoids, potentially generating indirect non-target effects on their native prey and host species (Holt & Hochberg, 2001; Davis *et al.*, 2006). Thus, improving predictability of effectiveness of biocontrol agents has much wider implications than contributing to improving control outcomes. Predicting agent effectiveness, which has been identified as a significant challenge, is slowly starting to increase use of weed population models, especially matrix models, which are emerging as robust tools.

Population dynamics of weeds in their invaded range are different to their dynamics in the native range (Sheppard, 2003), and are often different between parts of an invaded range (e.g. Shea *et al.*, 2005). Understanding what parameters in the life history of a weed drive its success in the invaded range is essential for designing effective control programmes. In the invaded range, weed population dynamics studies have two major roles:

- They help define the type and magnitude of the problem, against which the impacts of any control strategy, including biocontrol, could be evaluated; and they reveal the 'Achilles heel' – the weak point/s in the life cycle of the weed which, if targeted, would have the greatest effect on its population (e.g., Sheppard, 2003; Briese, 2006; Raghu *et al.*, 2006). For example, the matrix population model for *Carduus nutans* in New Zealand retrospectively determined that one of the most appropriate transitions has been targeted by the introduction of the receptacle

feeder *Rhinocyllus conicus* (Shea & Kelly, 1998) , albeit with an observed level of attack insufficient to reduce plant population density.

- Furthermore, they can assist in predicting the degree of pressure required at the weak point/s to achieve different control goals such as stopping weed populations from spreading or reducing weed density (Raghu *et al.*, 2006; Van Klinken & Raghu, 2006). A population model estimated, for example, that a reduction of 75% in seed production could reduce Scotch broom (*Cytisus scoparius* Link) abundance in Australia dramatically (Rees & Paynter, 1997); the population model for *C. nutans* in New Zealand, estimated that about 69% seed predation would be required for this thistle's populations to start declining (Shea & Kelly, 1998). The field estimates of only 30-40% seed predation by *R. conicus* (Kelly *et al.*, 1990; Kelly & McCallum, 1995) explained the failure by this agent to regulate the thistle. In Chapter 3 of this thesis I found levels of seed destruction achieved by *R. conicus* and *Urophora solstitialis* together were only about 23%; in Chapter 4.2 I found that a combined effect of *Trichosirocalus horridus* and *U. solstitialis* in New Zealand could reduce *C. nutans* seed production by at least 45%, in the absence of *R. conicus*. This is however still short of the 69% projected necessary by Shea and Kelly (1998).

This tightly links to predicting agent effectiveness and prioritising agent introduction. McClay and Balciunas (2005) list possible causes for ineffectiveness of agents which have successfully established and built up populations in the introduced range without having any effect on their target weed population. Some failures are associated with the part in the weed life cycle that is targeted by the agent; these include use of seed predators where weeds are not seed limited, use of herbivores that feed on tissues not essential for plant survival or reproduction, such as parenchyma and fruit pulp, and use of herbivores whose damage comes too late in the weed's phenology to have any significant effect on its growth or reproduction. Where population models are involved prior to agent introduction, agents that attack unimportant transitions or life stages are not considered (Briese, 2006; Davis *et al.*, 2006). It needs to be stressed that while weed population models from the native range offer valuable insights into limiting factors in the native range (Rees & Paynter, 1997; Jongejans *et al.*, 2006), they cannot replace models from the introduced range, where different transitions may be responsible for weed success. Once the weak points in the life cycle of the weed in its introduced range are revealed, the impact of potential agents are better off tested in the native range of the weed, prior to introduction (Sheppard, 2003; McClay & Balciunas, 2005).

Population models are very useful in estimating the combined effects of multiple biocontrol agent species in comparison to the individual effects of each species, and in estimating how this effect may differ with different sequences of agent introduction (Davis *et al.*, 2006). Clearly, this would be far more useful prior to agent introductions, yet, in this thesis, I have used Shea and Kelly's (1998) matrix population model for *C. nutans* in New Zealand, and the updated version (Jongejans *et al.*, 2008) retrospectively, and have estimated that the antagonistic effect of *R. conicus* on *U. solstitialis* potentially reduces *U. solstitialis* effectiveness (Chapter 4.1). I have also estimated that *U. solstitialis* and *T. horridus* together have a stronger adverse effect on *C. nutans* population growth rate than either species alone (Chapter 4.2). However, I was unable to estimate how the combined effect of the three agents would compare to their individual effects.

Models can be used to test the effects of combinations of control approaches and other stress factors on the weed population. Different stress factors may determine plant response to herbivory (Fowler & Rausher, 1985; Müller-Schärer , 1991). Land management use is likely to be important (Chapter 7). For example, where plant response to root herbivory resembles a response to water stress, well watered plants may compensate for herbivore damage, and no measurable effect would appear (Radcliff, 1971; Gange & Brown, 1989). Dairy pasture is intensively farmed, regularly irrigated and weeds are usually controlled chemically. In New Zealand it is quite plausible that neither biological nor chemical control affect *C. nutans* populations in dairy pasture; rather the strong competition from the healthy pasture may well be the dominant factor regulating this weed. This is a hypothesis that can be tested using matrix models, to improve management decisions in an active adaptive management framework. In an integrated management approach elsewhere, population models were able to indicate that the presence of biological control could be essential for the effectiveness of a combination of other control methods (mechanical and chemical in this case) (Buckley *et al.*, 2004).

Population models are perhaps the most practical tool we have today to predict biocontrol agents effectiveness and population-level effects of any management strategy. Their importance is slowly becoming realised (Shea, 2004; Raghu *et al.*, 2006) and they are increasingly used, both retrospectively, to evaluate past or ongoing biocontrol programmes (Lonsdale *et al.*, 1995; Shea & Kelly, 1998; Shea *et al.*, 2005; Shea *et al.*, 2006) and, more importantly (albeit rarely), prior to introduction of new agents (Davis *et al.*, 2006). Unfortunately, the potential of population models as a tool is better appreciated by ecologists than by funding agencies, which sees them largely underfunded or unfunded (Sheppard, 2003).

8.2 Sleeper weeds and multi targeting

Sleeper weeds have never received the attention they merit. This is mainly because weed biocontrol was, and in many cases still is, regarded as a last resort, and is employed only when conventional methods have failed to control the weed or have been controlling it unsustainably; when biological control is finally employed, the weed has already become a major problem (Thomas & Willis, 1998; Fowler *et al.*, 2000; Culliney, 2005; Ding *et al.*, 2006). Biocontrol opponents call for the problem caused by the weed to be well proven and for a high expected benefit-cost ratio to be demonstrated before a biocontrol agent is approved for release (Louda *et al.*, 1997; Strong, 1997; Louda *et al.*, 2003a). Sleeper weeds by definition fall short of complying with such requirements: they are not yet invasive, do not present an existing problem, and do not incur control costs. The understanding that prevention is far more efficient than cure has not yet sunk into environmental management. An exception has to be made regarding control against new introductions at the border in New Zealand, where immense efforts are taken to prevent unwanted organisms from entering the country; however, it also needs to be stressed that some of our current weeds and pests are organisms which have been introduced deliberately, and for which prevention at the border was waived. In addition, different strategies need to be developed for organisms which have escaped border control, and could still be prevented from becoming a problem, such as sleeper weeds.

New Zealand faces a risk, one of the most severe in the world, from invasive weeds: in less than 200 years of European settlement the flora here has increased more than tenfold, with more than 25,000 introduced plant species (Williams & Cameron, 2006). Not all introduced species would become invasive; but the number of naturalized exotic species (~2,200) already exceeds the number of natives (Williams & Cameron, 2006), and much of the country's exotic flora is considered to only be at the early stages of invasion (Sullivan *et al.*, 2005; Julien *et al.*, 2007). The list of invasive species already includes around 500 which are classified as weeds, of which about 200 are controlled by legislation (Julien *et al.*, 2007). Which of the 2,200 naturalized species will become invasive is difficult to predict (Williams & Lee, 2001; Sullivan *et al.*, 2004); in fact, vast but relatively unsuccessful efforts to characterise invasive species implies that robust generalizations are still a long way off (Rejmanek, 1999; Reichard, 2001; Rejmanek, 2001). The one generalization that can be made though, is that species that have become invasive in any introduced range are more likely to become invasive in other introduced areas (Reichard, 2001; Rejmanek, 2001). This one generalization should be used to prioritise research towards species that have become invasive elsewhere, and, if suitable for biological control, target these plants before they become a problem. No biocontrol agent is risk free (Sheppard *et al.*, 2003; Fowler & Withers, 2006);

yet, there are risks from not undertaking biocontrol, and the benefits from controlling sleeper weeds before they have become invasive can only be underestimated.

Multi-targeting as an approach to targeting sleeper weeds may attract resistance from biocontrol opponents on the grounds that it promotes non-target effects and promotes introduction of alien organisms to solve problems that do not exist. It needs to be emphasized therefore, that multi targeting is suggested as applicable for groups of plants that have no representatives in the native flora; hence, direct unwanted non-target effects are less likely in the first place (non-target species that are closely related to the target species are at much higher risk, Louda *et al.*, 2003b), and that it is suggested as part of biocontrol programmes against plants in these groups that are established weeds and already posing environmental or economic threats. Some examples of such plant groups in New Zealand are presented in Chapter 5. For at least one of the weedy species mentioned in Chapter 5,Japanese honeysuckle, *Lonicera japonica* Thunb., alien biocontrol agents are currently being searched for to initiate a classical biocontrol programme; S. V. Fowler, pers. Comm. Alien biocontrol agents are searched for, to be introduced against invasive representatives within these groups anyway; but, if less host-specific, potentially more effective, agents are considered, which could suppress other plants within the group, then fewer introductions of alien organisms could be expected in the long run, because: (a) the invasive species is more likely to be better suppressed by the potentially more effective agent; and (b) the potential future weeds are more likely to be maintained at non-damaging levels with no further agents required.

It is also warrant highlighting that New Zealand can proudly boast to employ one of the safest (if not **the** safest) systems in the world today for risk assessment in weed biocontrol (Sheppard *et al.*, 2003). It is only rational that a country which suffers one of the worst risks of invasive weeds and at the same time presents the best record for safety in classical biocontrol should embark on developing multi-targeting as a safe and effective novel strategy.

8.3 Patterns of herbivory on multiple plant species and multi targeting

The literature contains several contradicting theories, mechanisms and empirical evidence for the effect of the presence of one plant species on the degree of herbivory on another. One plant species could reduce herbivory on another, thus granting it ‘associational resistance’ by functioning as either an ‘insectory’ plant - maintaining predators and parasitoids which reduce herbivore density; a ‘repellent’ plant - repelling the herbivore, causing it to either fail to locate or to reject an appropriate host; or an ‘attractant-decoy’ plant - attracting the herbivore to an unsuitable plant which does not support its development (Atsatt & Odowd, 1976). These ideas

and mechanisms have been demonstrated in agricultural systems, where increasing plant diversity from monocultures to polycultures reduced herbivory damage to crop plants (e.g., Tahvanainen & Root, 1972; Parker, 1982). A preferred host may suffer increased herbivory when its relative density is increased (towards growing in pure stands) according to the 'resource concentration hypothesis' (Root, 1973; Risch, 1981). The mechanisms driving associational resistance and the resource concentration hypothesis greatly depend on the degree of specificity of the herbivore.

Associational resistance and the resource concentration response are highly relevant for management of horticultural systems, where monocultures are common; but these types of response would fail multi-targeting. For multi-targeting to succeed, interactions of the type of 'associational susceptibility' are required. Associational susceptibility occurs when the presence of one host plant species, usually a preferred host, inflicts **increased** herbivore damage on another, usually less preferred, acceptable plant host (Brown & Ewel, 1987). Associational susceptibility describes only the level of damage to the secondary host; if an effect on the secondary host survivorship or performance is demonstrated in addition, this becomes 'apparent competition' (Holt, 1977). Apparent competition is likely to be common in systems that present associational susceptibility but is a lot harder to demonstrate. In the system studied in this thesis associational susceptibility was observed, however, the system was not structured to test apparent competition, so it is not known whether any occurred (Chapter 5).

The impact of herbivores on the spatial distribution of hosts that do not form a significant part of their diet is possibly underestimated (Parker & Root, 1981; Rand, 2003). The presence of one host plant species has been demonstrated to restrict the distribution of another species through increased herbivory by a shared herbivore (Parker & Root, 1981; Rand, 2003). This indicates that associational susceptibility, if leading to apparent competition, could potentially play a significant role in limiting invasion by sleeper weeds.

Understanding the mechanisms driving associational susceptibility and the circumstances in which associational susceptibility may be expected could benefit target and host selection in biocontrol towards enhancing multi targeting. Some studies predicted associational susceptibility would be strictly linked to herbivore abundance and depletion of the preferred host, which forces the herbivore to transfer to the less preferred host (White & Whitham, 2000; Blossey *et al.*, 2001); Others (Russell *et al.*, 2007) have shown associational susceptibility occurs even when the preferred host is not depleted. Mechanisms suggested as possible drivers of associational susceptibility include **increased herbivore attraction** to patches where the preferred host is present, which represent high-quality patches (Hjalten *et*

al., 1993), and **'spillover' effect**, in which the preferred host is a source for high herbivore density, which spills onto the less preferred host (White & Whitham, 2000). Brown and Ewel (1987) suggested the palatability of a species relative to the other species with which it is associated would determine whether it would experience either associational resistance or associational susceptibility. However, it is quite likely that while the less palatable species suffers associational susceptibility, herbivory on the more palatable species remains unchanged. It would be undesirable in multi-targeting, that the preferred host (the target weed) would experience associational resistance by the presence of the less preferred host (the sleeper weed); hence, attack levels on both hosts should be tested when alone and when in combination.

One of the great challenges for effective practice of multi-targeting would be in identifying the suitable target weeds, sleeper weeds, and potential biocontrol agents. Holt and Hochberg (2001) listed three scenarios under which shared predation is likely to put non-target species in danger. These scenarios may assist identifying species combinations that are likely to be susceptible to multi targeting and are given here in multi targeting terms:

- The sleeper weed exhibits low intrinsic growth rate (it does not replenish loss easily)
- The biocontrol agent has high attack rates on the sleeper weed, even when it is sparse. This is likely for a biocontrol agent that does not become satiated easily.
- The target weed sustains the biocontrol agent in high abundance.

The final scenario is a key component in apparent competition, which is highly desirable in multi-targeting. Successful multi-targeting is highly likely by a biocontrol agent that attacks both the target and the sleeper weed heavily, and is sustained in high abundance by the target species.

Finally, while multi-targeting is aimed at preventing sleeper weeds from becoming invasive, it can also enhance biocontrol of the target weeds by sustaining biocontrol agent populations (even if only small), and sustaining constant pressure on the target weed, uncoupling the predator prey oscillations which normally characterise classical biocontrol systems, especially at early stages. Hence, at times when the preferred, target weed goes locally extinct, biocontrol agent populations could be maintained on the less preferred, sleeper weed, assuring a more rapid response when the target weed reinvades.

To conclude, a recurring theme throughout this thesis is that in complex multi-species systems, like classical biocontrol systems, less is in many respects more. The fewer agents

that get introduced, the lower the risk for competitive interactions which could jeopardise control efforts, and the lower the risk of indirect non-target effects in complex food-webs. Fewer agents with a relatively wide host range could target a wide range of potential (sleeper) weeds, again with lower risk for unwanted non-target effects involved, and with higher chances of success. However, an information gap of two decades has clearly demonstrated (Chapter 7) that more rather than less is necessary for long term monitoring of population level effects in biocontrol.

References

Agresti A. (1996). *An Introduction to Categorical Data Analysis*. John Wiley, New York, U.S.A. 290 pp.

Allan H.H. (1940). *A Handbook of the Naturalised Flora of New Zealand*. Government Printer, Wellington, New Zealand. 344 pp.

Alonso-Zarazaga M.A. & Sánchez-Ruiz M. (2002). Revision of the *Trichosirocalus horridus* (Panzer) species complex, with description of two new species infesting thistles (Coleoptera : Curculionidae, Ceutorhynchinae). *Australian Journal of Entomology*, **41**: 199-208.

Analytical Software (2003). *Statistix 8 User's Manual*. Analytical Software, Tallahassee, Florida. 396 pp.

Arnett A.E. & Louda S.M. (2002). Re-test of *Rhinocyllus conicus* host specificity, and the prediction of ecological risk in biological control. *Biological Conservation*, **106**: 251-257.

Atsatt P.R. & Odowd D.J. (1976). Plant defense guilds. *Science*, **193**: 24-29.

Auld B.A., Menz K.M. & Tisdell C.A. (1987). *Weed Control Economics*. Academic Press, London, UK. 177 pp.

Balciunas J.K. (2004). Are mono-specific agents necessarily safe? The need for pre-release assessment of probable impact of candidate biocontrol agents, with some examples. In: J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin & J.K. Scott, eds. Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO Publishing, Canberra, Australia. Pp 252–257

Bardgett R.D. & Wardle D.A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, **84**: 2258-2268.

Barratt B.I.P. & Moeed A. (2005). Environmental safety of biological control: policy and practice in New Zealand. *Biological Control*, **35**: 247-252.

Batcheler C.L. (1971). Estimation of density from a sample of joint point and nearest-neighbour distances. *Ecology*, **52**: 703-709.

Batcheler C.L. (1973). Estimating density and dispersion from truncated or unrestricted joint point-distance nearest-neighbour distances. *Proceedings of the New Zealand Ecological Society*, **20**: 131-147.

Batcheler C.L. (1975). Probable limit of error of the point distance-neighbour distance estimate of density. *Proceedings of the New Zealand Ecological Society*, **22**: 28-33.

Berube D.E. (1980). Interspecific competition between *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae) for ovipositional sites on diffuse knapweed (*Centaurea diffusa*, Compositae). *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology*, **90**: 299-306.

Bezemer T.M., Wagenaar R., Van Dam N.M. & Wackers F.L. (2003). Interactions between above- and belowground insect herbivores as mediated by the plant defense system. *Oikos*, **101**: 555-562.

Blossey B. (1995). A comparison of various approaches for evaluating potential biological control agents using insects on *Lythrum salicaria*. *Biological Control*, **5**: 113-122.

Blossey B., Casagrande R., Tewksbury L., Landis D.A., Wiedenmann R.N. & Ellis D.R. (2001). Nontarget feeding of leaf-beetles introduced to control purple loosestrife (*Lythrum salicaria* L.). *Natural Areas Journal*, **21**: 368-377.

Blossey B. & Hunt-Joshi T.R. (2003). Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annual Review of Entomology*, **48**: 521-547.

Boldt P.E. & Campobasso G. (1981). Biology of two weevils, *Ceutorhynchus trimaculatus* and *Trichosirocalus horridus* (Coleoptera: Curculionidae), on *Carduus* spp. (Compositae) in Europe. *Environmental Entomology*, **10**: 691-696.

Bonner K.I., Rahman A., James T.K., Nicholson K.S. & Wardle D.A. (1998). Relative intra-species competitive ability of nodding thistle biotypes with varying resistance to the herbicide 2, 4-D. *New Zealand Journal of Agricultural Research*, **41**: 291-297.

Bourdôt G.W., Fowler S.V., Edwards G.R., Kriticos D.J., Kean J.M., Rahman A. & Parsons A.J. (2007). Pastoral weeds in New Zealand: status and potential solutions. *New Zealand Journal of Agricultural Research*, **50**: 139-161.

Bourdôt G.W. & Kelly D. (1986). Density and cover estimates of some non-palatable herbaceous pasture weeds. *Proceedings of the New Zealand Weed and Pest Control Conference*, **39**: 183-186.

Briese D.T. (2006). Can an a priori strategy be developed for biological control? The case of *Onopordum* spp. thistles in Australia. *Australian Journal of Entomology*, **45**: 317-323.

Briese D.T., Sheppard A.W., Zwölfer H. & Boldt P.E. (1994). Structure of the phytophagous insect fauna of *Onopordum* thistles in the Northern Mediterranean basin. *Biological Journal of the Linnean Society*, **53**: 231-253.

Briese D.T., Thomann T. & Vitou J. (2002). Impact of the rosette crown weevil *Trichosirocalus briesei* on the growth and reproduction of *Onopordum* thistles. *Journal of Applied Ecology*, **39**: 688-698.

Briggs C.J. (1993). Competition among parasitoid species on a stage-structured host and its effect on host suppression. *American Naturalist*, **141**: 372-397.

Brown B.J. & Ewel J.J. (1987). Herbivory in complex and simple tropical successional ecosystems. *Ecology*, **68**: 108-116.

Brown V.K. & Gange A.C. (1989). Differential effects of above-ground and below-ground insect herbivory during early plant succession. *Oikos*, **54**: 67-76.

Brown V.K., Gange A.C., Evans I.M. & Storr A.L. (1987). The effect of insect herbivory on the growth and reproduction of two annual *Vicia* species at different stages in plant succession. *Journal of Ecology*, **75**: 1173-1189.

Buckley Y.M., Rees M., Paynter Q. & Lonsdale M. (2004). Modelling integrated weed management of an invasive shrub in tropical Australia. *Journal of Applied Ecology*, **41**: 547-560.

Cartwright B. & Kok L.T. (1985). Growth responses of musk and plumeless thistles (*Carduus nutans* and *Carduus acanthoides*) to damage by *Trichosirocalus horridus* (Coleoptera, Curculionidae). *Weed Science*, **33**: 57-62.

Caswell, H. (2001). *Matrix Population Models: Construction, Analysis and Interpertation*. 2nd ed. Sinauer Associates, Inc., Boston. 722 pp.

Chapin F.S. (1991). Integrated responses of plants to stress. *Bioscience*, **41**: 29-36.

Cockayne A.H. (1917). Noxious weeds in New Zealand. *New Zealand Journal of Agriculture*, **14**: 339-344.

Cousens R. & Mortimer M. (1995). *Dynamics of Weed Populations*. Cambridge University Press, Cambridge, UK. 332 pp.

Crawley M.J. (1988). Herbivores and plant population dynamics. In: *Plant Population Ecology* (A.J. Davy, M.J. Hutchings & A.R. Watkinson, eds.), pp. 367–392. Blackwell Scientific Publications, Oxford, UK.

Crawley M.J. (1989). Insect herbivores and plant population dynamics. *Annual Review of Entomology*, **34**: 531-564.

Crawley M.J. (1997). Plant-herbivore dynamics. In: *Plant Ecology*, pp. 401-474. Blackwell, London, UK.

Crooks J.A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, **97**: 153-166.

Crowe M.L. & Bourchier R.S. (2006). Interspecific interactions between the gall-fly *Urophora affinis* Frfld. (Diptera : Tephritidae) and the weevil *Larinus minutus* Gyll. (Coleoptera : Curculionidae), two biological control agents released against spotted knapweed, *Centaurea stobe* L. ssp. *micranthos*. *Biocontrol Science and Technology*, **16**: 417-430.

Culliney T.W. (2005). Benefits of classical biological control for managing invasive plants. *Critical Reviews in Plant Sciences*, **24**: 131-150.

Darwin C. (1860). *The Origin of Species by Means of Natural Selection*. 2nd edition. Murray, London, UK. 593 pp.

Davis A.S., Landis D.A., Nuzzo V., Blossey B., Gerber E. & Hinz H.L. (2006). Demographic models inform selection of biocontrol agents for garlic mustard (*Alliaria petiolata*). *Ecological Applications*, **16**: 2399-2410.

Delahunty E. (1960). Control of nodding thistle *Proceedings of the New Zealand Weed Control Conference*, **13**: 108-111.

Delahunty E. (1962). Nodding thistle. *Proceedings of the New Zealand Weed Control Conference*, **15**: 24-28.

Delfosse E.S. (2005). Risk and ethics in biological control. *Biological Control*, **35**: 319-329.

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

Denoth M., Frid L. & Myers J.H. (2002). Multiple agents in biological control: improving the odds? *Biological Control*, **24**: 20-30.

Ding J.Q., Blossey B., Du Y.Z. & Zheng F.S. (2006). Impact of *Galerucella birmanica* (Coleoptera: Chrysomelidae) on growth and seed production of *Trapa natans*. *Biological Control*, **37**: 338-345.

Dingwall A.R. (1962). The weeds of Canterbury. *Proceedings of the New Zealand Weed Control Conference*, **15**: 15-22.

Dowd P.F. & Kok L.T. (1983). Influence of soil conditions on *Carduus* thistles and development of the thistle head weevil, *Rhinocyllus conicus* (Coleoptera, Curculionidae). *Environmental Entomology*, **12**: 439-441.

Duncan R.P. & Williams P.A. (2002). Darwin's naturalization hypothesis challenged. *Nature*, **417**: 608-609.

Duncan R.P. & Young J.R. (2000). Determinants of plant extinction and rarity 145 years after European settlement of Auckland, New Zealand. *Ecology*, **81**: 3048-3061.

Dunn P.H. & Rizza A. (1976). Bionomics of *Psylliodes chalcomera*, a candidate for biological control of musk thistle. *Annals of the Entomological Society of America*, **69**: 395-398.

Edmonds D.K. & Popay A.I. (1983). Effect of pasture competition on the survival and flowering of nodding thistle. *Proceedings of the New Zealand Weed and Pest Control Conference*, **36**: 89-92.

Environment Canterbury (2006). Canterbury Regional Pest Management Strategy (2005-2015): operational plan for 2006-07. Environment Canterbury, Christchurch, New Zealand. 43 pp.

Evans L., Grimes A., Wilkinson B. & Teece D. (1996). Economic reform in New Zealand 1984-95: the pursuit of efficiency. *Journal of Economic Literature*, **34**: 1856-1902.

Feldman I., McCarty M.K. & Scifers C.J. (1968). Ecological and control studies of musk thistle. *Weed Science*, **16**: 1-4.

Fowler N.L. & Rausher M.D. (1985). Joint effects of competitors and herbivores on growth and reproduction in *Aristolochia reticulata*. *Ecology*, **66**: 1580-1587.

Fowler S.V., Gourlay A.H., Hill R.H. & Withers T.M. (2004). Safety in New Zealand weed biocontrol: a retrospective analysis of host-specificity testing and the predictability of impacts on non-target plants. In: J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin & J.K. Scott, eds. Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO Publishing, Canberra, Australia. Pp 265-270

Fowler S.V., Memmot J., Paynter Q.E., W. S.A. & Syrett P. (2001). The scope and value of extensive ecological studies in the broom biological control programme. In: *Evaluating Indirect Ecological Effects of Biological Control* (E. Wajnberg, J.K. Scott & P.C. Quimby, eds.), pp. 229-248. CABI Publishing, Wallingford, UK.

Fowler S.V., Syrett P. & Hill R.L. (2000). Success and safety in biological control of environmental weeds in New Zealand. *Austral Ecology*, **25**: 553-562.

Fowler S.V. & Withers T.M. (2006). Biological control: reducing the impact of invasive weeds and pests, or just another source of alien invaders. In: *Biological Invasions in New Zealand* (R. Allen & W.G. Lee, eds.), pp. 355-369. Springer, Berlin, Germany.

Frank J.H. (1998). How risky is biological control? Comment. *Ecology*, **79**: 1829-1834.

Freidberg A. (1984). Gall Tephritidae (Diptera). In: *Biology of Gall Insects* (T.N. Ananthakrishnan, ed.), pp. 129–167. Edward Arnold, London, UK.

Futuyma D.J. & Wasserman S.S. (1980). Resource concentration and herbivory in oak forests. *Science*, **210**: 920-922.

Gange A.C. & Brown V.K. (1989). Effects of root herbivory by an insect on a foliar-feeding species, mediated through changes in the host plant. *Oecologia*, **81**: 38-42.

Gassman A. (2005). Classification of thistle species and potential for biological control of *Cirsium arvense* in New Zealand. Special Report, CABI Bioscience, CABI, Delémont, Switzerland. 25 pp.

Gassmann A. & Louda S.M. (2001). *Rhinocyllus conicus*: initial evaluation and subsequent ecological impacts in North America. In: *Evaluating Indirect Ecological Effects of Biological Control. CABI Publishing, Wallingford* (E. Wajnberg, J.K. Scott & P.C. Quimby, eds.), pp. 147–183. CABI Publishing, Wallingford, UK.

Gerber E., Hinz H.L. & Blossey B. (2007a). Impact of the belowground herbivore and potential biological control agent, *Ceutorhynchus scrobicollis*, on *Alliaria petiolata* performance. *Biological Control*, **42**: 355-364.

Gerber E., Hinz H.L. & Blossey B. (2007b). Interaction of specialist root and shoot herbivores of *Alliaria petiolata* and their impact on plant performance and reproduction. *Ecological Entomology*, **32**: 357-365.

Goeden R.D. (1974). Comparative survey of phytophagous insect faunas of Italian thistle, *Cardus pycnocephalus*, in southern California and southern Europe relative to biological weed control. *Environmental Entomology*, **3**: 464-474.

Goeden R.D. (1988). A capsule history of biological control of weeds. *Biocontrol News and Information*, **9**: 55-61.

Goeden R.D. & Kok L.T. (1986). Comments on a proposed 'new' approach for selecting agents for the biological control of weeds. *Canadian Entomologist*, **118**: 51-58.

Goeden R.D. & Ricker D.W. (1977). Establishment of *Rhinocyllus conicus* on milk thistle in southern California. *Weed Science*, **25**: 288-292.

Goeden R.D. & Ricker D.W. (1978). Establishment of *Rhinocyllus conicus* (Col: Curculionidae) on Italian thistle in southern California. *Environmental Entomology*, **7**: 787-789.

Goeden R.D. & Ricker D.W. (1985). Seasonal asynchrony of Italian thistle, *Carduus pycnocephalus*, and the weevil, *Rhinocyllus conicus* (Coleoptera: Curculionidae), introduced for biological control in southern California. *Environmental Entomology*, **14**: 433-436.

Goldson S.L., Bourdôt G.W. & Proffitt J.R. (1987). A study of the effects of *Sitona discoideus* (Coleoptera: Curculionidae) larval feeding on the growth and development of lucerne (*Medicago sativa*). *Journal of Applied Ecology*, **24**: 153-161.

Green R.H. (1979). *Sampling Design and Statistical Methods for Environmental Biologists*. John Wiley, New York, U.S.A. 257 pp.

Groenteman R., Kelly D., Fowler S.V. & Bourdôt G.W. (2007). Interactions between nodding thistle seed predators. *New Zealand Plant Protection*, **60**: 152-157.

Groenteman R., Kelly D., Fowler S.V. & Bourdôt G.W. (in press a). Factors affecting oviposition preference in the weevil *Rhinocyllus conicus* (Froehlich). In: M.H. Julien & R. Sforza, eds. Proceedings of the XII International Symposium on Biological Control of Weeds, La Grande Motte, France.

Groenteman R., Kelly D., Fowler S.V. & Bourdôt G.W. (in press b). Which species of the thistle biocontrol agent *Trichosirocalus* are present in New Zealand? In: M.H. Julien & R. Sforza, eds. Proceedings of the XII International Symposium on Biological Control of Weeds, La Grande Motte, France.

Groves R.H. & Kaye P.E. (1989). Germination and phenology of seven introduced thistle species in Southern Australia. *Australian Journal of Botany*, **37**: 351-359.

Guretzky J.A. & Louda S.M. (1997). Evidence for natural biological control: insects decrease survival and growth of a native thistle. *Ecological Applications*, **7**: 1330-1340.

Haefliger P., Schwarzlaender M. & Blossey B. (2006). Impact of *Archanara geminipuncta* (Lepidoptera: Noctuidae) on aboveground biomass production of *Phragmites australis*. *Biological Control*, **38**: 413-421.

Hairston N.G., Smith F.E. & Slobodkin L.B. (1960). Community structure, population control, and competition. *American Naturalist*, **94**: 421-425.

Harman H.M., Syrett P., Hill R.L. & Jessep C.T. (1996). Arthropod introductions for biological control of weeds in New Zealand, 1929-1995. *New Zealand Entomologist*, **19**: 71-80.

Harrington K.C., Popay A.I., Robertson A.G. & McPherson H.G. (1988). Resistance of nodding thistle to MCPA in Hawkes Bay. *Proceedings of the New Zealand Weed and Pest Control Conference*, **41**: 219-222.

Harris P. (1973). The selection of effective agents for biological control of weeds. *Canadian Entomologist*, **105**: 1495-1503.

Harris P. (1981). Stress as a strategy in the biological control of weeds. *Beltsville Symposia in Agricultural Research*, **5**.

Harris P. (1984). *Carduus nutans* L., nodding thistle and *C. acanthoides* L., plumeless thistle (Compositae). In: *Biological Control Programs Against Insects and Weeds in Canada 1969-1980* (J.S. Kelleher & M.A. Hume, eds.), pp. 115-126. Commonwelth Agricultural Bureaux Farnham Royal, UK.

Harris P. (1989a). Feeding strategy, coexistance and impact of insects in spotted knapweed capitula. In: E.S. Delfosse, ed. proceedings of the VII International Symposium on biological control of weeds, Rome, Italy. Pp 39-47

Harris P. (1989b). The use of Tephritidae for the biological control of weeds. *Biological Control News and Information*, **10**: 7–16.

Harris P. (1991). Classical biocontrol of weeds - its definition, selection of effective agents, and administrative political problems. *Canadian Entomologist*, **123**: 827-849.

Harris P. & Shorthouse J.D. (1996). Effectiveness of gall inducers in weed biological control. *Canadian Entomologist*, **128**: 1021-1055.

Hartley M.J. (1983). Effects of Scotch thistles on sheep growth rates. *Proceedings of the New Zealand Weed and Pest Control Conference*, **36**: 86-89.

Hawaiian Ecosystems at Risk project & AgWest (2007). Global Compendium of Weeds. Date accessed: 20 October 2007. URL: http://www.hear.org/gcw/

Hayes L. (2007). Nodding thistle gall fly *Urophora solstitialis*. Date accessed: 12 November 2007. URL: http://www.landcareresearch.co.nz/research/biocons/weeds/book/documents/Nodding_Thistle_Gall_Fly.pdf

Headrick D.H. & Goeden R.D. (1998). The biology of nonfrugivorous tephritid fruit flies. *Annual Review of Entomology*, **43**: 217-241.

Hjalten J., Danell K. & Lundberg P. (1993). Herbivore avoidance by association - vole and hare utilization of woody plants. *Oikos*, **68**: 125-131.

Hoffmann J.H. & Moran V.C. (1992). Oviposition patterns and the supplementary role of a seed feeding weevil, *Rhyssomatus marginatus* (Coleoptera: Curculionidae), in the biological

control of a perennial leguminous weed, *Sesbania punicea*. *Bulletin of Entomological Research*, **82**: 343-347.

Holland P. & Rahman A. (1999). Review of trends in agricultural pesticide use in New Zealand. MAF Policy Technical Paper. 53 pp.

Holt R.D. (1977). Predation, apparent competition, and structure of prey communities. *Theoretical Population Biology*, **12**: 197-229.

Holt R.D. & Hochberg M.E. (2001). Indirect interactions, community modules and biological control: a theoretical perspective. In: *Evaluating Indirect Ecological Effects of Biological Control* (E. Wajnberg, J.K. Scott & P.C. Quimby, eds.), pp. 13–37. CABI Publishing, Wallingford, UK.

Howarth F.G. (1983). Classical biocontrol: panacea or Pandora's box. *Proceedings of Hawaiian Entomological Society*, **24**: 239-244.

Howarth F.G. (1991). Environmental impacts of classical biological control. *Annual Review of Entomology*, **36**: 485-509.

Huffaker C.B. (1959). Biological control of weeds with insects. *Annual Review of Entomology*, **4**: 251-276.

Huffaker C.B. & Kennett C.E. (1969). Some aspects of assessing eficiency of natural enemies. *Canadian Entomologist*, **101**: 425-447.

Hunt-Joshi T.R., Blossey B. & Root R.B. (2004). Root and leaf herbivory on *Lythrum salicaria*: implications for plant performance and communities. *Ecological Applications*, **14**: 1574-1589.

Hurrell G.A., Bourdôt G.W. & Saville D.J. (2001). Effect of application time on the efficacy of Sclerotinia sclerotiorum as a mycoherbicide for Cirsium arvense control in pasture. *Biocontrol Science and Technology*, **11**: 317-330.

Janzen D.H. (1971). Seed predation by animals. *Annual Review of Ecology and Systematics*, **2**: 465-492.

Jessep C.T. (1975). Introduction of a weevil for the biological control of nodding thistle. *proceedings of New Zealand Weed and Pest Control Conference 28*: 205-206.

Jessep C.T. (1989a). *Carduus nutans* L., nodding thistle (Asteraceae). In: *A Review of Biological Control of Invertebrate Pests and Weeds in New Zealand 1874 to 1987* (P.J. Cameron, R.L. Hill, J. Bain & W.P. Thomas, eds.), pp. 339-342. CAB International, Wallingford, U.K.

Jessep C.T. (1989b). Introduction of the crown weevil (*Trichosirocalus horridus*) as an additional biocontrol agent against nodding thistle. *Proceedings of New Zealand Weed and Pest Control Conference* **42**: 52-54.

Jessep C.T. (1990). Aspects of the biology of nodding thistle (*Carduus nutans* L.) in Canterbury, New-Zealand. *New Zealand Journal of Agricultural Research*, **33**: 173-183.

Jongejans E., Shea K., Skarpaas O., Kelly D., Sheppard A. W. & Woodburn T. (2008). Dispersal and demography contributions to population spread of *Carduus nutans* in its native and invaded ranges. *Journal of Ecology*, **96:** 687-697.

Jongejans E., Sheppard A.W. & Shea K. (2006). What controls the population dynamics of the invasive thistle *Carduus nutans* in its native range? *Journal of Applied Ecology*, **43**: 877-886.

Jordon-Thaden I.E. & Louda S.M. (2003). Chemistry of *Cirsium* and *Carduus*: a role in ecological risk assessment for biological control of weeds? *Biochemical Systematics and Ecology*, **31**: 1353-1396.

Julien M.H. & Griffiths M.W. (1998). *Biological control of weeds, Fourth edition: A world catalogue of agents and their target weeds*. CABI Publishing, Wallingford, U.K. 223 pp.

Julien M.H., Scott J.K., Orapa W. & Paynter Q. (2007). History, opportunities and challenges for biological control in Australia, New Zealand and the Pacific islands. *Crop Protection*, **26**: 255-265.

Kelly D. (1988). Demography of *Carduus pycnocephalus* and *C. tenuiflorus*. *New Zealand Natural Sciences*, **15**: 17-24.

Kelly D. & McCallum K. (1990). Demography, seed biology and biological control of *Carduus nutans* in New Zealand. In: J.P. Palmer, ed. The Biological Control of Invasive Plants: Proceedings of a Conference of the British Ecological Society. British Ecological Society, Cardiff. Pp 72-79

Kelly D. & McCallum K. (1995). Evaluating the impact of *Rhinocyllus conicus* on *Carduus nutans* in New Zealand. In: E.S. Delfosse & R.R. Scott, eds. Proceedings of the VIII International Symposium on Biological Control of Weeds. Pp 205-212

Kelly D., McCallum K., Schmidt C.J. & Scanlan P.M. (1990). Seed predation in nodding and slender winged thistles by nodding thistle receptacle weevil. *Proceedings of New Zealand Weed and Pest Control Conference* **43**: 212-215.

Kelly D. & Popay A.I. (1985). Pasture production lost to unsprayed thistles at two sites. *Proceedings of the New Zealand Weed and Pest Control Conference*, **38**: 115-118.

Kelly D. & Wood R. (1991). Why nodding thistle receptacle weevil destroys so little nodding thistle seed in Canterbury. *Proceedings of New Zealand Weed and Pest Control Conference* **44**: 280-283.

Kirk T. (1899). *The Students' Flora of New Zealand and the Outlying Islands*. Government Printer, Wellington, New Zealand. 408 pp.

Klein M. & Seitz A. (1994). Geographic differentiation between populations of *Rhinocyllus conicus* Frolich (Coleoptera: Curculionidae): concordance of allozyme and morphometric analysis. *Zoological Journal of the Linnean Society*, **110**: 181-191.

Kok L.T. (1978). Biological control of *Carduus* thistles in northeastern U.S.A. In: T.E. Freeman, ed. Proceedings of the IV International Symposium on Biological Control of Weeds, Gainesville. Pp 101-104

Kok L.T. (1986). Impact of *Trichosirocalus horridus* (Coleoptera, Curculionidae) on *Carduus* thistles in pastures. *Crop Protection*, **5**: 214-217.

Kok L.T. & Mays W.T. (1991). Successful biological control of plumeless thistle, *Carduus acanthoides* L. [Campanulatae: Asteraceae (= Compositae)], by *Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae) in Virginia. *Biological Control*, **1**: 197-202.

Kok L.T. & McAvoy T.J. (1983). Refrigeration, a practical technique for storage of eggs of *Trichosirocalus horridus* (Coleoptera: Curculionidae). *Canadian Entomologist*, **115**: 1537-1538.

Kok L.T. & Surles W.W. (1975). Successful biocontrol of musk thistle Campanulatae: Compositae by an introduced weevil, *Rhinocyllus conicus* Coleoptera: Curculionidae. *Environmental Entomology*, **4**: 1025-1027.

Lambdon P.W. & Hulme P.E. (2006). Predicting the invasion success of Mediterranean alien plants from their introduction characteristics. *Ecography*, **29**: 853-865.

Lamp W.O. & McCarty M.K. (1982a). Biology of pre-dispersal seed predators of the platte thistle, *Cirsium canescens*. *Journal of the Kansas Entomological Society*, **55**: 305-316.

Lamp W.O. & McCarty M.K. (1982b). Pre-dispersal seed predation of a native thistle, *Cirsium canescens*. *Environmental Entomology*, **11**: 847-851.

Lonsdale W.M., Farrell G. & Wilson C.G. (1995). Biological control of a tropical weed: a population model and experiment for *Sida acuta*. *Journal of Applied Ecology*, **32**: 391-399.

Louda S.M. (1982a). Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. *Ecological Monographs*, **52**: 25-41.

Louda S.M. (1982b). Limitation of the recruitment of the shrub *Haplopappus squarrosus* (Asteraceae) by flower- and seed-feeding insects. *Journal of Ecology*, **70**: 43-53.

Louda S.M. & Arnett A.E. (2000). Predicting non-target ecological effects of biological control agents: evidence from *Rhinocyllus conicus*. In: N.R. Spencer, ed. Proceedings of the X International Symposium on Biological Control of Weeds. Agricultural Research Service Montana, Bozeman. Pp 551-567

Louda S.M., Arnett A.E., Rand T.A. & Russell F.L. (2003a). Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. *Conservation Biology*, **17**: 73-82.

Louda S.M., Kendall D., Connor J. & Simberloff D. (1997). Ecological effects of an insect introduced for the biological control of weeds. *Science*, **277**: 1088-1090.

Louda S.M., Pemberton R.W., Johnson M.T. & Follett P.A. (2003b). Nontarget effects - The Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology*, **48**: 365-396.

Louda S.M. & Potvin M.A. (1995). Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. *Ecology*, **76**: 229-245.

Louda S.M., Potvin M.A. & Collinge S.K. (1990). Predispersal seed Predation, postdispersal seed predation and competition in the recruitment of seedlings of a native thistle in sandhills prairie. *American Midland Naturalist*, **124**: 105-113.

Louda S.M., Rand T.A., Russell F.L. & Arnett A.E. (2005). Assessment of ecological risks in weed biocontrol: input from retrospective ecological analyses. *Biological Control*, **35**: 253-264.

Louda S.M. & Rodman J.E. (1996). Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). *Journal of Ecology*, **84**: 229-237.

Louda S.M. & Stiling P. (2004). The double-edged sword of biological control in conservation and restoration. *Conservation Biology*, **18**: 50-53.

Macdonald I.A.W. (2004). Recent research on alien plant invasions and their management in South Africa: a review of the inaugural research symposium of the Working for Water programme. *South African Journal of Science*, **100**: 21-26.

Maron J.L. (1998). Insect herbivory above- and belowground: individual and joint effects on plant fitness. *Ecology*, **79**: 1281-1293.

Maron J.L. (2001). Intraspecific competition and subterranean herbivory: individual and interactive effects on bush lupine. *Oikos*, **92**: 178-186.

Martin P. & Rahman A. (1988a). Emergence of nodding thistle seedlings in pasture at two sites in the Waikato. *Proceedings of the New Zealand Weed and Pest Control Conference*, **41**: 210-213.

Martin P. & Rahman A. (1988b). Influence of sheep grazing on the survival and fate of nodding thistle rosettes. *Proceedings of the New Zealand Weed and Pest Control Conference*, **41**: 214-218.

Masters G.J. (1995a). The effect of herbivore density on host plant mediated interactions between two insects. *Ecological Research*, **10**: 125-133.

Masters G.J. (1995b). The impact of root herbivory on aphid performance - field and laboratory evidence. *Acta Oecologica-International Journal of Ecology*, **16**: 135-142.

Masters G.J. & Brown V.K. (1992). Plant-mediated interactions between two spatially separated insects. *Functional Ecology*, **6**: 175-179.

Masters G.J., Brown V.K. & Gange A.C. (1993). Plant mediated interactions between aboveground and belowground insect herbivores. *Oikos*, **66**: 148-151.

Masters G.J., Jones T.H. & Rogers M. (2001). Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. *Oecologia*, **127**: 246-250.

Mattson W.J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**: 119-161.

McCarty M.K. (1982). Musk thistle (*Carduus thoermeri*) seed production. *Weed Science*, **30**: 441-445.

McCarty M.K. & Lamp W.O. (1982). Effect of a weevil, *Rhinocyllus conicus*, on musk thistle (*Carduus thoermeri*) seed production. *Weed Science*, **30**: 136-140.

McClay A.S. (1992). Beyond 'before-and-after': experimental design and evaluation in classical weed biocontrol. In: E.S. Delfosse & R.R. Scott, eds. Proceedings of the VIII International Symposium on Biological Control of Weeds. CSIRO, Melbourne, Australia, Lincoln, New Zealand. Pp 213-219

McClay A.S. & Balciunas J.K. (2005). The role of pre-release efficacy assessment in selecting classical biological control agents for weeds-applying the Anna Karenina principle. *Biological Control*, **35**: 197-207.

McEvoy P., Cox C. & Coombs E. (1991). Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications*, **1**: 430-442.

McEvoy P.B. & Coombs E.M. (1999). Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecological Applications*, **9**: 387-401.

McEvoy P.B. & Coombs E.M. (2000). Why things bite back: unintended consequences of biological weed control. In: *Nontarget Effects of Biological Control.* (P.A. Follett & J.J. Duan, eds.), pp. 167-194. Kluwer Academic Publishers, Boston, Massachusetts, U.S.A.

McEvoy P.B., Rudd N.T., Cox C.S. & Huso M. (1993). Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. *Ecological Monographs*, **63**: 55-75.

McFadyen R.E. (2003). Does ecology help in the selection of biocontrol agents. In: J.H. Spafford & D.T. Briese, eds. Improving the Selection, Testing and Evaluation of Weed Biological Control Agents. Cooperative Research Centre for Australian Weed Management, Adelaide, Australia. Pp 5–9

McFadyen R.E. & Weggler-Beaton K. (2000). The biology and host specificity of *Liothrips* sp. (Thysanoptera : phlaeothripidae), an agent rejected for biocontrol of annual ragweed. *Biological Control*, **19**: 105-111.

McFadyen R.E.C. (1998). Biological control of weeds. *Annual Review of Entomology*, **43**: 369-393.

McNeill M.R. & Fletcher C.J. (2005). Interspecific competition between *Rhinocyllus conicus* and *Urophora solstitialis* L. on nodding thistle in Canterbury? *New Zealand Plant Protection*, **58**: 140-147.

Medd R.W. (1977). Some Aspects of the Ecology and Control of *Carduus nutans* (Nodding Thistle) on the Northern Tablelands, N.S.W. Unpublished PhD Thesis. University of New England, Armidale, New South Wales, Australia. 209 pp.

Mgidi T.N., Le Maitre D.C., Schonegevel L., Nel J.L., Rouget M. & Richardson D.M. (2007). Alien plant invasions - incorporating emerging invaders in regional prioritization: a pragmatic approach for Southern Africa. *Journal of Environmental Management*, **84**: 173-187.

Milbrath L.R. & Nechols J.R. (2004a). Indirect effect of early-season infestations of *Trichosirocalus horridus* on *Rhinocyllus conicus* (Coleoptera : Curculionidae). *Biological Control*, **30**: 95-109.

Milbrath L.R. & Nechols J.R. (2004b). Individual and combined effects of *Trichosirocalus horridus* and *Rhinocyllus conicus* (Coleoptera : Curculionidae) on musk thistle. *Biological Control*, **30**: 418-429.

Moeller-Joop H. & Schroeder D. (1986). *Urophora solstitialis* L. (Diptera: Tephritidae) a candidate for biological control of plumeless thistle (*Carduus acanthoides* L.) in Canada. Final screening report C.A.B. 23 pp.

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

Moran V.C., Hoffmann J.H. & Zimmermann H.G. (2005). Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. *Frontiers in Ecology and the Environment*, **3**: 77-83.

Morin L. & Edwards P.B. (2006). Selection of biological control agents for bridal creeper: a retrospective review. *Australian Journal of Entomology*, **45**: 287-291.

Morón-Rios A., Dirzo R. & Jaramillo V.J. (1997). Defoliation and below-ground herbivory in the grass *Muhlenbergia quadridentata*: effects an plant performance and on the root-feeder *Phyllophaga* sp. (Coleoptera: Melolonthidae). *Oecologia*, **110**: 237-242.

Müller-Schärer H. (1991). The impact of root herbivory as a function of plant density and competition: survival, growth and fecundity of *Centaurea maculosa* in field plots. *Journal of Applied Ecology*, **28**: 759-776.

Müller-Schärer H. & Brown V.K. (1995). Direct and indirect effects of aboveground and belowground insect herbivory on plant density and performance of *Tripleurospermum perforatum* during early plant succession. *Oikos*, **72**: 36-41.

Myers J.H. (1985). How many insect species are necessary for successful biocontrol of weeds. In: E.S. Delfosse, ed. proceedings of the VI International Symposium on biological control of weeds. Agriculture Canada. Canadian Govt. Printing Office, Ottawa, Canada, Vancouver, Canada. Pp 77-82

Myers J.H. & Harris P. (1980). Distribution of *Urophora* galls in flower heads of diffuse and spotted knapweed in British Columbia. *Journal of Applied Ecology*, **17**: 359-367.

Newingham B.A., Callaway R.M. & BassiriRad H. (2007). Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory. *Oecologia*, **153**: 913-920.

NIWA (2007). Climate Summary for June 2006. Date accessed: 3rd December 2007. URL: http://www.niwa.co.nz/ncc/cs/monthly/ma/mclimsum_06_06

Nötzold R., Blossey B. & Newton E. (1998). The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia*, **113**: 82-93.

Olckers T. (2004). Targeting emerging weeds for biological control in South Africa: the benefits of halting the spread of alien plants at an early stage of their invasion. *South African Journal of Science*, **100**: 64-68.

Parker M.A. (1982). Association with mature plants protects seedlings from predation in an arid grassland shrub, *Gutierrezia microcephala*. *Oecologia*, **53**: 276-280.

Parker M.A. & Root R.B. (1981). Insect herbivores limit habitat distribution of a native composite, *Machaeranthera canescens*. *Ecology*, **62**: 1390-1392.

Paynter Q.E., Fowler S.V., Gourlay A.H., Haines M.L., Harman H.M., Hona S.R., Peterson P.J., Smith L.A., Wilson-Davey J.R.A., Winks C.J. & Withers T.M. (2004). Safety in New Zealand weed biocontrol: a nationwide survey for impacts on non-target plants. *New Zealand Plant Protection*, **57**: 102-107.

Pemberton R.W. (2000). Predictable risk to native plants in weed biological control. *Oecologia*, **125**: 489-494.

Persson P.I. (1963). Studies on the biology and larval morphology of some Trypetidae (Dipt.). *Opuscula Entomologica*, **28**: 33-69.

Phung H.T. & Popay A.I. (1981). Effect of pasture cover on the germination of certain weed seeds. *Proceedings of the New Zealand Weed and Pest Control Conference*, **34**: 111-113.

Pomerinke M.A., Thompson D.C. & Clason D.L. (1995). Bionomics of *Cleonidius trivittatus* (Coleoptera: Curculionidae): native biological control of purple locoweed (Rosales: Fabaceae). *Environmental Entomology*, **24**: 1696-1702.

Popay A.I. & Kelly D. (1986). Seasonality of emergence, and survival, of nodding thistle. *Proceedings of the New Zealand Weed and Pest Control Conference*, **39**: 187-191.

Popay A.I., Lyttle L.A., Edmonds D.K. & Phung H.T. (1984). Incidence of the nodding thistle receptacle weevil on nodding and slender winged thistles. *Proceedings of New Zealand Weed and Pest Control Conference*, **37**: 28-32.

Popay A.I. & Medd R.W. (1990). The biology of Australian weeds 21. *Carduus nutans* L. ssp. *nutans*. *Plant Protection Quarterly*, **5**: 3-13.

Popay A.I., Mitchell R.B. & Thompson A. (1979). Age, size and fate of nodding thistles. *Proceedings of the New Zealand Weed and Pest Control Conference*, **32**: 21-26.

Prins A.H., Nell H.W. & Klinkhamer P.G.L. (1992). Size-dependent root herbivory on *Cynoglossum officinale*. *Oikos*, **65**: 409-413.

R Development Core Team (2006). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria.

Radcliff J.E. (1971). Effects of grass grub (*Costelytra zealandica* White) larvae on pasture plants. 2. Effect of grass grubs and soil moisture on perennial ryegrass and cocksfoot. *New Zealand Journal of Agricultural Research*, **14**: 607-&.

Raghu S. & van Klinken R.D. (2006). Refining the ecological basis for agent selection in weed biological control. *Australian Journal of Entomology*, **45**: 251-252.

Raghu S., Wilson J.R. & Dhileepan K. (2006). Refining the process of agent selection through understanding plant demography and plant response to herbivory. *Australian Journal of Entomology*, **45**: 308-316.

Rand T.A. (1999). Effects of environmental context on the susceptibility of *Atriplex patula* to attack by herbivorous beetles. *Oecologia*, **121**: 39-46.

Rand T.A. (2003). Herbivore-mediated apparent competition between two salt marsh forbs. *Ecology*, **84**: 1517-1526.

Rand T.A. & Louda S.M. (2004). Exotic weed invasion increases the susceptibility of native plants attack by a biocontrol herbivore. *Ecology*, **85**: 1548-1554.

Rand T.A. & Louda S.M. (2006). Invasive insect abundance varies across the biogeographic distribution of a native host plant. *Ecological Applications*, **16**: 877-890.

Rees M. & Brown V.K. (1992). Interactions between invertebrate herbivores and plant competition. *Journal of Ecology*, **80**: 353-360.

Rees M. & Paynter Q. (1997). Biological control of Scotch broom: modelling the determinants of abundance and the potential impact of introduced insect herbivores. *Journal of Applied Ecology*, **34**: 1203-1221.

Rees N.E. (1977). Impact of *Rhinocyllus conicus* (Coleoptera: Curculionidae) on thistles in southwestern Montana. *Environmental Entomology*, **6**: 839-842.

Reichard S. (2001). The search for patterns that enable prediction of invasion. In: *Weed Risk Assessment* (R.H. Groves, F.D. Panetta & J.G. Virtue, eds.), pp. 10-19. CSIRO Publishing, Collingwood, Australia.

Rejmanek M. (1999). Invasive plant species and invasible ecosystems. In: *Invasive Species and Biodiversity Management* (O.T. Sandlund, P.J. Schei & Å. Viken, eds.), pp. 79-102. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rejmanek M. (2001). What tools do we have to detect invasive species? In: *Weed Risk Assessment* (R.H. Groves, F.D. Panetta & J.G. Virtue, eds.), pp. 3-9. CSIRO Publishing, Collingwood, Australia.

Richardson D.M., Pysek P., Rejmanek M., Barbour M.G., Panetta F.D. & West C.J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, **6**: 93-107.

Risch S.J. (1981). Insect herbivore abundance in tropical monocultures and polycultures - an experimental test of two hypotheses. *Ecology*, **62**: 1325-1340.

Rizza A., Campobasso G., Dunn P.H. & Stazi M. (1988). *Cheilosia corydon* (Diptera: Syrphidae), a candidate for the biological control of musk thistle in North America. *Annals of the Entomological Society of America*, **81**: 225-232.

Roduner M.A., Cuperus G., Mulder P., Stritzke J. & Payton M. (2003). Successful biological control of the musk thistle in Oklahoma using the musk thistle head weevil and the rosette weevil. *American Entomologist*, **49**: 112-120.

Root R.B. (1973). Organization of a plant-arthropod association in simple and diverse habitats: fauna of collards (Brassica Oleracea). *Ecological Monographs*, **43**: 95-120.

Roy B., Popay A.I., Champion P., James T.K. & Rahman A. (2004). *An Illustrated Guide to Common Weeds of New Zealand*. 2nd edition. New Zealand Plant Protection Society, Lincoln, New Zealand. 314 pp.

Russell F.L., Louda S.M., Rand T.A. & Kachman S.D. (2007). Variation in herbivore-mediated indirect effects of an invasive plant on a native plant. *Ecology*, **88**: 413-423.

Salt D.T., Fenwick P. & Whittaker J.B. (1996). Interspecific herbivore interactions in a high CO_2 environment: root and shoot aphids feeding on *Cardamine*. *Oikos*, **77**: 326-330.

Seastedt T.R., Ramundo R.A. & Hayes D.C. (1988). Maximization of densities of soil animals by foliage herbivory: empirical evidence, graphical and conceptual models. *Oikos*, **51**: 243-248.

Sezen Z. (2007). Interactions of the Invasive Thistle *Carduus nutans* and its Biocontrol Agent *Rhinocyllus conicus* in Heterogeneous Environments. Unpublished Ph.D. Thesis. Pennsylvania State University, University Park, PA, USA. 163 pp.

Shea K. (2004). Models for improving the targeting and implementation of biological control of weeds. *Weed Technology*, **18**: 1578-1581.

Shea K. & Kelly D. (1998). Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecological Applications*, **8**: 824-832.

Shea K. & Kelly D. (2004). Modeling for management of invasive species: musk thistle (*Carduus nutans*) in New Zealand. *Weed Technology*, **18**: 1338-1341.

Shea K., Kelly D., Sheppard A.W. & Woodburn T.L. (2005). Context-dependent biological control of an invasive thistle. *Ecology*, **86**: 3174-3181.

Shea K., Possingham H.P., Murdoch W.W. & Roush R. (2002). Active adaptive management in insect pest and weed control: intervention with a plan for learning. *Ecological Applications*, **12**: 927-936.

Shea K., Sheppard A. & Woodburn T. (2006). Seasonal life-history models for the integrated management of the invasive weed nodding thistle *Carduus nutans* in Australia. *Journal of Applied Ecology*, **43**: 517-526.

Sheppard A.W. (2003). Prioritising agents based on predicted efficacy: beyond the lottery approach. In: J.H. Spafford & D.T. Briese, eds. Improving the Selection, Testing and Evaluation of Weed Biological Control Agents. Cooperative Research Centre for Australian Weed Management, Adelaide, Australia. Pp 11-22

Sheppard A.W., Aeschlimann J.P., Sagliocco J.L. & Vitou J. (1991). Natural enemies and population stability of the winter-annual *Carduus pycnocephalus* L. in Mediterranean Europe. *Acta Oecologica*, **12**: 707-726.

Sheppard A.W., Aeschlimann J.P., Sagliocco J.L. & Vitou J. (1995). Belowground herbivory in *Carduus nutans* (Asteraceae) and the potential for biological control. *Biocontrol Science and Technology*, **5**: 261-270.

Sheppard A.W., Cullen J.M. & Aeschlimann J.P. (1994). Predispersal seed predation on *Carduus nutans* (Asteraceae) in southern Europe. *Acta Oecologica*, **15**: 529-541.

Sheppard A.W., Cullen J.M., Aeschlimann J.P., Sagliocco J.L. & Vitou J. (1990). The importance of insect herbivores relative to other limiting factors on weed population dynamics: a case study of *Carduus nutans*. In: E.S. Delfosse, ed. proceedings of the VII International Symposium on biological control of weeds, Rome, Italy. Pp 211-219

Sheppard A.W., Hill R., DeClerck-Floate R.A., McClay A., Olckers T., Quimby Jr P.C. & Zimmermann H.G. (2003). A global review of risk-benefit-cost analysis for the introduction of classical biological control agents against weeds: a crisis in the making? *Biocontrol News and Information*, **24**: 91-108.

Sheppard A.W., Smyth M.J. & Swirepik A. (2001). The impact of a root-crown weevil and pasture competition on the winter annual *Echium plantagineum*. *Journal of Applied Ecology*, **38**: 291-300.

Sheppard A.W. & Woodburn T. (1996). Population regulation in insects used to control thistles: can this predict effectiveness? In: *Frontiers of Population Ecology* (R.B. Floyd, A.W. Sheppard & P.J. De Barro, eds.), pp. 277-290. CSIRO publishing, Melbourne.

Sieburth P.J. & Kok L.T. (1982). Ovipositional preference of *Trichosirocalus horridus* (Coleoptera: Curculionidae). *Canadian Entomologist*, **114**: 1201-1202.

Sieburth P.J., Kok L.T. & Lentner M. (1983). Factors influencing the effectiveness of *Trichosirocalus horridus* (Panzer) in the control of *Carduus* thistles. *Crop Protection*, **2**: 143-151.

Simberloff D. & Stiling P. (1996a). How risky is biological control? *Ecology*, **77**: 1965-1974.

Simberloff D. & Stiling P. (1996b). Risks of species introduced for biological control. *Biological Conservation*, **78**: 185-192.

Simberloff D. & Stiling P. (1998). How risky is biological control? Reply. *Ecology*, **79**: 1834-1836.

Smith L.M., II & Kok L.T. (1987). Influence of temperature on oviposition, quiescence, and mortality of *Rhinocyllus conicus* (Coleoptera: Curculionidae). *Environmental Entomology*, **16**: 971-974.

Soler R., Bezemer T.M., Cortesero A.M., Van der Putten W.H., Vet L.E.M. & Harvey J.A. (2007). Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia*, **152**: 257-264.

Standish R.J. (2001). Prospects for Biological Control of *Tradescantia fluminensis* Vell. (Commelinaceae). Science Internal Series 9, Department of Conservation, Wellington, New Zealand. 25 pp.

Stanley M.C. & Fowler S.V. (2004). Conflicts of interests associated with the biological control of weeds. In: J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin & J.K. Scott, eds. Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO Publishing, Canberra, Australia. Pp 322-340

Steinger T. & Müller-Schärer H. (1992). Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil-nitrogen availability. *Oecologia*, **91**: 141-149.

Straw N.A. (1989). The timing of oviposition and larval growth by two tephritid fly species in relation to host plant development. *Ecological Entomology*, **14**: 443-454.

Strong D.R. (1997). Fear no weevil? *Science*, **277**: 1058-1059.

Strong D.R., Maron J.L., Connors P.G., Whipple A., Harrison S. & Jefferies R.L. (1995). High mortality, fluctuation in numbers, and heavy subterranean insect herbivory in bush lupine, *Lupinus arboreus*. *Oecologia*, **104**: 85-92.

Strong D.R. & Pemberton R.W. (2000). Biological control of invading species - risk and reform. *Science*, **288**: 1969-1970.

Sullivan J.J., Timmins S.M. & Williams P.A. (2005). Movement of exotic plants into coastal native forests from gardens in northern New Zealand. *New Zealand Journal of Ecology*, **29**: 1-10.

Sullivan J.J., Williams P.A., Cameron E.K. & Timmins S.M. (2004). People and time explain the distribution of naturalized plants in New Zealand. *Weed Technology*, **18**: 1330-1333.

Surles W.W. & Kok L.T. (1978). *Carduus* thistle seed destruction by *Rhinocyllus conicus*. *Weed Science*, **26**: 264-269.

Syrett P., Harman H.M. & Fowler S.V. (1995). Identification of risk to kowhai, a New Zealand native plant *Sophora microphylla* Ait., from a potential biological control agent for broom, *Cytisus scoparius* (L.) Link. *New Zealand Journal of Zoology*, **22**: 305-309.

Tahvanainen J.O. & Root R.B. (1972). Influence of vegetational diversity on population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera Chrysomelidae). *Oecologia*, **10**: 321-&.

Thomas C.D. (1986). Butterfly larvae reduce host plant survival in vicinity of alternative host species. *Oecologia*, **70**: 113-117.

Thomas M.B. & Willis A.J. (1998). Biocontrol - risky but necessary? *Trends in Ecology & Evolution*, **13**: 325-329.

Thomas P.A. & Room P.M. (1986). Taxonomy and control of *Salvinia molesta*. *Nature*, **320**: 581-584.

Thompson A., Saunders A.E. & Martin P. (1987). The effect of nodding thistle (*Carduus nutans*) on pasture production. *Proceedings of the New Zealand Weed and Pest Control Conference*, **40**: 222-225.

Tipping P.W. (1992). The impact of three insect herbivores on seed production of musk thistle (*Carduus thoermeri*). *Maryland Entomologist*, **3**: 155-159.

Turnbull L.A., Crawley M.J. & Rees M. (2000). Are plant populations seed-limited? A review of seed sowing experiments. *Oikos*, **88**: 225-238.

Turner C.E. (1996). Tephritidae in biological control of weeds. In: *Fruit Fly Pests: A World Assessment of Their Biology and Management* (B.A. McPheron & S.G. J., eds.), pp. 157-164. St. Lucie Press, Delray Beach, Florida, U.S.A.

Turner C.E., Pemberton R.W. & Rosenthal S.S. (1987). Host utilization of native *Cirsium* thistles (Asteraceae) by the introduced weevil *Rhinocyllus conicus* (Coleoptera: Curculionidae) in California. *Environmental Entomology*, **16**: 111-115.

Van der Putten W.H., Vet L.E.M., Harvey J.A. & Wackers F.L. (2001). Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution*, **16**: 547-554.

Van Klinken R.D. & Raghu S. (2006). A scientific approach to agent selection. *Australian Journal of Entomology*, **45**: 253-258.

Wahl M. & Hay M.E. (1995). Associational resistance and shared doom - effects of epibiosis on herbivory. *Oecologia*, **102**: 329-340.

Wapshere A.J. (1974). Strategy for evaluating safety of organisms for biological weed control. *Annals of Applied Biology*, **77**: 201-211.

Wardle D.A., Nilsson M.C., Gallet C. & Zackrisson O. (1998). An ecosystem-level perspective of allelopathy. *Biological Reviews*, **73**: 305-319.

Warren W.G. & Batcheler C.L. (1979). The density of spatial patterns: robust estimation through distance methods. In: *Statistical Ecology* (R.M. Cormack & J.K. Ord, eds.), pp. 247-270. International Co-operative Publishing House, Fairland, Maryland, U.S.A.

Webb C.J., Sykes W.R., Garnock-Jones P.J. & Given D.R. (1988). *Flora of New Zealand. Volume IV, Naturalised Pteridophytes, Gymnosperms, Dicotyledons*. Botany Division D.S.I.R., Christchurch, N.Z. 1365 pp.

White J.A. & Whitham T.G. (2000). Associational susceptibility of cottonwood to a box elder herbivore. *Ecology*, **81**: 1795-1803.

Williams J.A. & West C.J. (2000). Environmental weeds in Australia and New Zealand: issues and approaches to management. *Austral Ecology*, **25**: 425-444.

Williams P.A. & Cameron E.K. (2006). Creating gardens:the diversity and progression of European plant introductions. In: *Biological Invasions in New Zealand* (R. Allen & W.G. Lee, eds.), pp. 33-47. Springer, Berlin, Germany.

Williams P.A. & Lee W.G. (2001). Why screen for weediness? *New Zealand Garden Journal*, **4**: 19-23.

Woodburn T.L. (1996a). Interspecific competition between *Rhinocyllus conicus* and *Urophora solstitialis*, two biocontrol agents released in Australia against *Carduus nutans*. In: V.C. Moran & J.H. Hoffmann, eds. Proceedings of the IX International Symposium on Biological Control of Weeds, Stellenbosch, South Africa. Pp 409-415

Woodburn T.L. (1996b). Reduction of seed set in nodding thistle (*Carduus nutans*) by the seed-fly, *Urophora solstitialis*, in Australia. In: *Fruit Fly Pests: A World Assessment of Their Biology and Management* (B.A. McPheron & G.J. Steck, eds.), pp. 165-169. St. Lucie Press, Delray Beach, Florida.

Woodburn T.L. (1997). Establishment in Australia of *Trichosirocalus horridus* a biological control agent for *Carduus nutans*, and preliminary assessment of its impact on plant growth and reproductive potential. *Biocontrol Science and Technology*, **7**: 645-656.

Woodburn T.L. & Briese D.T. (1996). The contribution of biological control to the management of thistles. *Plant Protection Quarterly.*, **11**: 250-253.

Woodburn T.L. & Cullen J.M. (1996). Impact of *Rhinocyllus conicus* and *Urophora solstitialis* on seed set in *Carduus nutans* in Australia. In: N.J. Hind, ed. Proceedings of the International Compositae Conference, Kew, UK. Pp 307-319

Woodburn T.L. & Sheppard A.W. (1995). *Urophora solstitialis*, a potential biological control agent for *Carduus nutans* in Australia. In: E.S. Delfosse & R.R. Scott, eds. Proceedings of the VIII Symposium on Biological Control of Weeds. CSIRO Publishing, Melbourne, Australia, Lincoln, New Zealand. Pp 415

Zwölfer H. (1965). Preliminary list of phytophagous insects attacking wild Cynareae (Compositae) in Europe. *Technical Bulletin of the Commonwealth Institute for Biological Control*, **6**: 88-154.

Zwölfer H. (1973). Competition and coexistance in phytophagous insects attacking the heads of *Carduus nutans* L. In Proceedings of the II International Symposium on Biological Control of Weeds. Pp 74-77

Zwölfer H. & Harris P. (1971). Host specificity determination of insects for biological control of weeds. *Annual Review of Entomology*, **16**: 159-&.

Zwölfer H. & Harris P. (1984). Biology and host specificity of *Rhinocyllus conicus* (Froel) (Col: Curculionidae), a successful agent for biocontrol of the thistle, *Carduus nutans* L. *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology*, **97**: 36-62.

Zwölfer H. & Preiss M. (1983). Host selection and oviposition behavior in west European ecotypes of *Rhinocyllus conicus* Froel (Col: Curculionidae). *Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology*, **95**: 113-122.

Appendix A: Supplementary statistical analysis for Section 4.1

The differences in insect counts (log transformed) between treatments were compared in mixed effect models in R (R Development Core Team, 2006), with Poisson distributions, using the 'lmer' command. All relevant explanatory variables were included in the models as covariates. Non-categorical covariates were standardized (i.e., divided by the variance after subtraction of the mean); this made the magnitudes of their effects comparable, despite being measured in different units. Non-significant variables were excluded from the models by backwards selection, using the Chi-squared test to compare between models. Only the best-fit minimum models are presented. When testing each biocontrol agent's final density, the treatments not including that agent had to be excluded from the analysis to avoid upsetting the model (which would result in infinite standard error terms). Plants, plots and blocks were included in the models as random grouping factors. The 'lmer' command automatically corrects for overdispersion in the data if required. The model outputs are presented as tables of parameter estimates, in which the intercept estimate includes the lowest level of each categorical variable (in an alphabetic or numeric order), and all following estimates appear as the **difference** from the intercept; the z-test for each parameter tests the null hypothesis that this **difference** is not statistically different from zero. Categorical variable were labelled such that the alphabetical or numerical value would offer a logical order of testing.

Difference in the number (log transformed) per capitulum of *U. solstitalis* individuals at low (five pairs) and high (15 pairs) initial densities, and in the presence of zero, low (one pair) and high (three pairs) initial densities of *R. conicus*: generalised linear mixed effect models with Poisson distribution and with plants, plots (cages) and blocks as grouping factors. Based on 583 capitula from 130 plants in 30 plots (cages) and five blocks.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	-7.367	1.021	-7.216	<0.001
U.s. high	1	2.033	1.155	1.760	0.078
U.s. low R.c. low	1	-2.401	1.432	-1.676	0.094
U.s. high R.c. low	1	1.799	1.202	1.498	0.134
U.s. low R.c.high	1	-0.802	1.242	-0.645	0.519
U.s. high R.c.high	1	1.395	1.152	1.211	0.226
Leaf length at the time of bolting	1	-0.982	0.426	-2.304	0.021
Plant heigth	1	2.113	0.503	4.201	<0.001
Date collected	1	-3.267	0.296	-11.048	<0.001
External diametr	1	0.978	0.097	10.058	<0.001
Capitulum height	1	-0.678	0.258	-2.625	0.009
Capitulum position	1	1.945	0.513	3.789	<0.001

[1]Intercept: *U. solstitialis* low initial density, collected on 11 Jan 2006, at a primary position

Difference in the final number (log transformed) per capitulum of *R. conicus* individuals at low (one pairs) and high (three pairs) initial densities, and in the presence of zero, low (five pair) and high (15 pairs) initial densities of *U. solstitalis*: generalised linear mixed effect models with Poisson distribution and with plants, plots (cages) and blocks as grouping factors. Based on 655 capitula from 136 plants in 30 plots (cages) and five blocks.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept[1]	1	-3.063	0.491	-6.244	<0.001
R.c.high	1	2.381	0.594	4.011	<0.001
R.c. low U.s. low	1	0.842	0.652	1.293	0.196
R.c. low U.s. high	1	2.050	0.638	3.214	0.001
R.c.high U.s. low	1	2.518	0.619	4.066	<0.001
R.c.high U.s. high	1	2.195	0.613	3.579	<0.001
Date collected	1	-1.284	0.075	-17.161	<0.001
External diametr	1	0.143	0.048	2.968	0.003
Capitulum height	1	0.230	0.093	2.465	0.014
Capitulum position	1	-0.911	0.203	-4.489	<0.001

[1]Intercept: *R. conicus* low initial density, collected on 11 Jan 2006, at a primary position

Appendix B: Supplementary material for chapter 5

Tables 1, 2, 3 and 4 correspond with Tables 5.3, 5.4, 5.5 and 5.6 in Chapter 5, respectively. The analysis of *C. pycnocephalus* and *C. tenuiflorus* in Chapter 5 was done on a dataset which excluded two extreme replicates; the analysis presented in Tables 1, 2 and 3 below followed the same statistical procedures, on the dataset that includes those extreme replicates.

Table 1: Difference in capitulum's probability of attack by *R. conicus* (logit transformed) in the presence and absence of *C. nutans* in (**a**) *C. pycnocephalus* and (**b**) *C. tenuiflorus*: generalized linear mixed effects models with a binomial distribution and with plants, plots and blocks as grouping factors; *C. pycnocephalus* and *C. tenuiflorus* were only tested before mid December. (a). *C. pycnocephalus*. Based on 1537 capitula from 117 plants. Intercept - *C. nutans* absent, primary cluster position.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.375	0.214	-1.75	0.080
C. nutans present	1	0.178	0.303	0.589	0.556
External diameter	1	0.281	0.067	4.191	<0.001
Cluster position - secondary	1	-0.977	0.157	-6.237	<0.001
Leaf length at the time of bolting	1	-0.523	0.151	-3.456	<0.001
Plant height in mm	1	0.459	0.198	2.316	0.021
Sum of clusters on the plant	1	2.186	0.506	4.321	<0.001
Sum of capitula on the plant	1	-1.224	0.458	-2.671	0.008
Sum of capitula in the plot	1	-1.227	0.337	-3.642	<0.001
Average plot height in mm	1	0.747	0.277	2.7	0.007

(b). *C. tenuiflorus*. Based on 1484 capitula from 115 plants. Intercept - *C. nutans* absent, bolted by 26 Sep., 2005.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.803	0.240	-3.342	<0.001
C. nutans present	1	-0.069	0.299	-0.230	0.818
External diameter	1	0.188	0.072	2.612	0.009
Capitulum height in mm	1	-0.499	0.089	-5.610	<0.001
Sum of capitula in the cluster	1	0.441	0.068	6.490	<0.001
Plant height in mm	1	0.556	0.113	4.915	<0.001
Bolted between 26 Sep. and 6 Oct.	1	-0.170	0.197	-0.860	0.390
Bolted between 6 Oct. and 21 Oct.	1	-0.766	0.310	-2.471	0.013
Bolted between 21 Oct. and 6 Dec.	1	-0.182	0.952	-0.192	0.848

Table 2: Difference in the number of *R. conicus* attack signs per capitulum (log transformed) in the presence and absence of *C. nutans* in (**a**) *C. pycnocephalus* and (**b**) *C. tenuiflorus*: generalized linear mixed effects models with a Poisson distribution and with plants, plots and blocks as grouping factors; *C. pycnocephalus* and *C. tenuiflorus* were only tested before mid December. (a). *C. pycnocephalus*. Based on 1537 capitula from 117 plants. Intercept - *C. nutans* absent, primary cluster position.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-0.788	0.175	-4.515	<0.001
C. nutans present	1	0.269	0.247	1.090	0.276
External diameter	1	0.244	0.043	5.700	<0.001
Capitulum height in mm	1	-0.092	0.042	-2.210	0.027
Cluster position - secondary	1	-0.504	0.102	-4.929	<0.001
Leaf length at the time of bolting	1	-0.197	0.090	-2.177	0.029
Sum of clusters on the plant	1	1.905	0.380	5.019	<0.001
Sum of capitula on the plant	1	-1.111	0.338	-3.289	0.001
Sum of capitula in the plot	1	-1.200	0.251	-4.786	<0.001
Average plot height in mm	1	0.947	0.196	4.842	<0.001

(b). *C. tenuiflorus*. Based on 1499 capitula from 117 plants. Intercept - *C. nutans* absent.

Fixed effects					
	df	Estimate	Std. Error	z value	*P*
Intercept	1	-1.186	0.159	-7.452	<0.001
C. nutans present	1	0.037	0.226	0.164	0.869
External diameter	1	0.141	0.052	2.742	0.006
Capitulum height in mm	1	-0.372	0.056	-6.597	<0.001
Sum of capitula in the cluster	1	0.264	0.045	5.881	<0.001
Standardized plant height in mm	1	0.416	0.083	4.993	<0.001

Table 3: Difference in the cumulative proportion (logit transformed) of (**a**) *C. pycnocephalus* and (**b**) *C. tenuiflorus* capitula (the total processed) exhibiting increasing rank of *R. conicus* feeding damage in the presence and absence of *C. nutans*: proportional odds (cumulative logit) models. The model is interpreted as a series of regression lines (one for each cut point); to model capitula in the absence of *C. nutans*, the slope term for '*C. nutans* present' is omitted.

(a). *C. pycnocephalus*. Based on 1539 capitula.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
C. nutans present	1	-0.110	0.172	-0.660	0.509
Sum of *R. conicus* signs	1	1.380	0.081	17.030	<0.001
Secondary position	1	-0.470	0.234	-2.010	0.045
Sum of clusters on the plant	1	-0.880	0.350	-2.520	0.012
Sum of capitula on the plant	1	1.100	0.332	3.320	0.001
Intercepts for cut points of damage categories					
	df	Estimate	Std. Error	t value	*P*
No damage (0.147, 0.176)[1]	1	2.066	0.132	15.680	<0.001
Little damage (0.135, 0.164)	1	2.204	0.134	16.419	<0.001
Medium damage (0.096, 0.103)	1	2.870	0.148	19.353	<0.001

(b). *C. tenuiflorus*. Based on 1485 capitula.

Fixed effects					
	df	Estimate	Std. Error	t value	*P*
C. nutans present	1	-0.15	0.18	-0.87	0.384
Sum of *R. conicus* signs	1	2.03	0.11	18.78	<0.001
Bolted between 26 Sep. and 6 Oct.	1	-0.32	0.19	-1.70	0.089
Bolted between 6 Oct. and 21 Oct.	1	-0.70	0.34	-2.07	0.039
Bolted between 21 Oct. and 6 Dec.	1	-0.63	0.95	-0.66	0.509
Sum of capitula in the plot	1	-0.52	0.18	-2.87	0.004
Average plot height in mm	1	0.65	0.18	3.56	<0.001
Intercepts for cut points of damage categories					
	df	Estimate	Std. Error	t value	*P*
No damage (0.188, 0.231)	1	1.77	0.16	10.84	<0.001
Little damage (0.175, 0.210)	1	1.98	0.17	11.96	<0.001
Medium damage (0.142, 0.158)	1	2.55	0.17	14.69	<0.001

[1]Numbers in parentheses beside each cut point for damage category are the cumulative proportions of capitula exhibiting higher damage rating than that cut point in the presence and absence of *C. nutans* respectively.

Table 4: Difference in the percentage of (**a**) *C. pycnocephalus* and (**b**) *C. tenuiflorus* capitula attacked by *R. conicus* (of the total processed) and the proportion of capitula exhibiting actual *R. conicus* feeding damage (of those that got attacked) early in the season (before 13 December) in the presence and absence of *C. nutans*. The percentages of damaged capitula of those attacked are also compared between early and late season (regardless of *C. nutans* presence). Sub-samples from the bulk collections were either excluded or included.

(a). *C. pycnocephalus*

	Bulk collections excluded			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	39.1 (675)	37.5 (264)	Early season	39.6 (636)
Early season, *C. nutans* absent	42.8 (870)	41.1 (372)	Late season	56.0 (216)
χ^2	2.09	0.85		17.61
P	0.149	0.357		<0.001

	Bulk collections included			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	37.7 (1045)	40.9 (394)	Early season	41.0 (943)
Early season, *C. nutans* absent	39.2 (1402)	41.2 (549)	Late season	53.9 (414)
χ^2	0.54	0.01		19.12
P	0.491	0.926		<0.001

(b). *C. tenuiflorus*

	Bulk collections excluded			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	29.7 (741)	63.2 (220)	Early season	66.2 (473)
Early season, *C. nutans* absent	33.5 (761)	68.8 (253)	Late season	59.0 (200)
χ^2	2.53	1.64		3.14
P	0.112	0.200		0.076

	Bulk collections included			
	% attacked (N)	% damaged of attacked (N)		% damaged of attacked (N)
Early season, *C. nutans* present	29.5 (1167)	63.9 (344)	Early season	66.8 (739)
Early season, *C. nutans* absent	29.4 (1349)	69.4 (395)	Late season	62.3 (329)
χ^2	0.00	2.43		2.07
P	0.989	0.119		0.150

The numbers of capitula collected (and processed), attacked and damaged by *R. conicus* which were used to construct Table 4 above and Table 5.6 in Chapter 5, are presented in Table 5 below.

Table 5: Number of non-target species capitula collected (and processed), number attacked by *R. conicus*, and number exhibited *R. conicus* feeding damage.

(a). *C. pycnocephalus*

All replicates included							
Bulk collections excluded	Collected	Attacked	Damaged		Collected	Attacked	Damaged
C. nutans absent	870	372	153	Early	1545	636	252
C. nutans present	675	264	99	Late	3284	216	121
Bulk collections included							
C. nutans absent	1402	549	226	Early	2447	943	387
C. nutans present	1045	394	161	Late	8899	414	223
Two replicates excluded							
Bulk collections excluded							
C. nutans absent	526	205	73	Early	976	410	154
C. nutans present	450	205	81	Late	2298	152	81
Bulk collections included							
C. nutans absent	927	342	124	Early	1609	617	236
C. nutans present	682	275	112	Late	6374	313	158

(b). *C. tenuiflorus*

All replicates included							
Bulk collections excluded	Collected	Attacked	Damaged		Collected	Attacked	Damaged
C. nutans absent	761	255	174	Early	1500	473	313
C. nutans present	741	220	139	Late	3299	200	118
Bulk collections included							
C. nutans absent	1349	397	274	Early	2514	739	494
C. nutans present	1167	344	220	Late	9637	329	205
Two replicates excluded							
Bulk collections excluded							
C. nutans absent	430	147	98	Early	887	308	204
C. nutans present	458	162	106	Late	2078	164	97
Bulk collections included							
C. nutans absent	860	246	171	Early	1605	486	323
C. nutans present	746	241	152	Late	6470	265	168

Table 5 (contd)
(c). *C. vulgare*

	Collected	Attacked	Damaged
C. nutans absent	190	7	6
C. nutans present	116	10	9

Table 6: Attack by *R. conicus* and *U. solstitialis* on non-target species, at sites with *C. nutans* either present or absent at the field surveys in 2005-06 and 2006-07.
(a). *C. tenuiflorus*

			2005-06		2006-07	
	Site	*C. nutans* present	Un-attacked	Attacked	Un-attacked	Attacked
R. conicus	B. Peninsula	no	102	0	97	3
	Culverden	yes	75	25	74	24
U. solstitalis	B. Peninsula	no	102	0	100	0
	Culverden	yes	100	0	93	5
Either	B. Peninsula	no	102	0	97	3
	Culverden	yes	75	25	70	28

(b). *C. pycnocephalus*

			2006		2007	
	Site	*C. nutans* present	Un-attacked	Attacked	Un-attacked	Attacked
R. conicus	B. Peninsula	no	100	2	99	1
	Culverden	yes	94	6	82	18
U. solstitalis	B. Peninsula	no	102	0	100	0
	Culverden	yes	100	0	100	0
Either	B. Peninsula	no	100	2	99	1
	Culverden	yes	94	6	82	18

(c). *C. vulgare*

			2006		2007	
	Site	*C. nutans* present	Un-attacked	Attacked	Un-attacked	Attacked
R. conicus	B. Peninsula	no	73	26	83	17
	Culverden	yes	47	53	77	23
	Rakaia Gorge	no	83	18	76	24
	Rakaia Gorge	yes	87	4	77	24

Appendix C: Multiple species density estimation using the truncated joint point-distance nearest-neighbour distance method

Densities were calculated for each region as a whole; paddocks were not replicates.

Stage 1: estimation of thistle density in the region (globally, combined for all species):

Let:

N = the global number of random points = 62 paddocks x 30 points per paddock = 1860 (in this example).
p = the number of points that had a plant within the search radius.
r_p = the distance from the random point to the nearest plant.
n = the number of nearest neighbours.
r_n = the distance from the first plant to the nearest neighbour.
m = the number of next nearest neighbours.
r_m = the distance from the nearest neighbour to the next nearest neighbour.
R = the search radius in meters.

Then:

1. $f = \frac{p}{N}$ frequency of points that had a plant

2. $d = \frac{p}{\pi(\Sigma r_p^2 + (N-p)R^2)}$ density estimator for a randomly distributed population

Let:

E(CV) = the expected coefficient of variation of r_p, calculated from:

$\log_e E(CV) = -1.0319 + o.4892 f^2 - 0.7182 f^4 + 0.6095 f^6$ (see Batcheler, 1973)

Then:

3. $A_1 = \frac{1}{E(CV)} \sqrt{\frac{\left(p\Sigma r_p^2 - \left(\Sigma r_p\right)^2\right)n^2 N}{\Sigma r_p \Sigma r_n p^3}}$ an index of non-randomness

4. $A_2 = \frac{1}{E(CV)} \sqrt{\frac{\left(p\Sigma r_p^2 - \left(\Sigma r_p\right)^2\right)m^2 N}{\Sigma r_p \Sigma r_m p^2 n}}$ an index of non-randomness

5. $\log_e E(CV) = -1.03107 + 0.48924 f^2 - 0.71817 f^4 + 0.60946 f^6$

6. $a = 1 + 2.473 f$ intercept for the dependant regression of bias on the index of non-randomness (A)

7. $b = 1 + 2.717\,f$ slope for the dependant regression of bias on the index of non-randomness (A)

8. $\lambda_{Global} = 10^4 \times \frac{d}{2a}\left(b^{A1} + b^{A2}\right)$ corrected regional weed density (plants per ha)

Stage 2: Estimate the individual contribution of each species to the global density:

Let:

v_i = the number of pairs of the species i (i.e., pairs in which p and n are the same species and pairs in which n and m are the same species).
r_{ni} = the distance between pairs of plants in which p and n are the same species
r_{mi} = the distance between pairs of plants in which n and m are the same species

Then:

9. $L = \frac{\Sigma r_n + \Sigma r_m}{n + m}$ mean distance between all pairs of plants

10. $l_i = \frac{r_{ni} + r_{mi}}{v_i}$ mean distance between pairs of the same species

11. $C_i = \frac{L}{l_i} \times \frac{p_i + n_i + m_i}{p + n + m}$ contribution of the species i to the regional density

12. $\lambda_i = \frac{C_i}{\Sigma C_i} \times \lambda_{Global}$ density of the species i

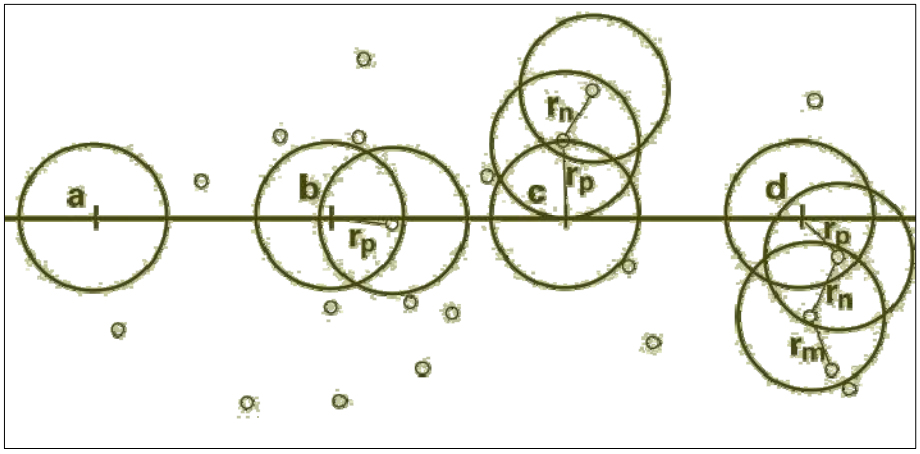

Figure 1: An illustration of the truncated point-distance nearest-neighbour technique, adapted from Batcheler (1975), showing the quantities rp, rn and rm. In the example illustrated here N = 4, p = 3, n = 2 and m = 1.

Trichosirocalus horridus on *Cirsium vulgare*

"…if well though out methods of repression... are generally adopted the weed problem should in time disappear." A.H. Cockayne, 1917, on noxious weeds in New Zealand