Understanding the Implications of Size Difference in Nodding Thistle Crown Weevil, *Trichosirocalus horridus*, for the Successful Biological Control of Nodding Thistle (*Carduus nutans*)

Ronny Groenteman

Trichosirocalus horridus

Understanding the Implications of Size Difference in Nodding Thistle Crown Weevil, *Trichosirocalus horridus*, for the Successful Biological Control of Nodding Thistle (*Carduus nutans*)

Ronny Groenteman

Landcare Research
PO Box 40, Lincoln 7640
New Zealand

Landcare Research Contract Report: LC0910/119

PREPARED FOR:
Horizons Regional Council

DATE: April 2010

Reviewed by:	Approved for release by:
Quentin Paynter Scientist Landcare Research	Matt McGlone Science Team Leader Biodiversity and Conservation

Contents

Summary 5
1. Introduction.. 7
2. Background.. 7
3. Objectives 8
4. Methods 8
   4.1 Thistle collection 8
   4.2 Analysis.. 8
   4.3 Weevil collection.. 9
   4.4 Statistical analysis 9
5. Results.. 9
   5.1 Geographic distribution of the survey 9
   5.2 Size of adult weevils 12
   5.3 Rosette size and larval counts 14
   5.4 Nutrients 17
6. Conclusions.. 19
7. Recommendations.. 20
8. Acknowledgements.. 20
9. References.. 20
   Appendix 1 Nodding thistle crown sampling sheet 21

Summary

Project and Client

A study to compare the size of a biocontrol agent, nodding thistle crown weevil (*Trichosirocalus horridus*), between different regions of New Zealand, and the relationship with nitrogen and phosphorus levels in the host plant, was undertaken for Horizons Regional Council between July 2009 and April 2010. The aim of the study was to see if the performance of this agent could be improved in the Manawatu-Wanganui Region, where it has been suggested that populations are low and provide inadequate control. This project was funded by an Envirolink medium advice grant (HZLC73).

Objectives

- Assess whether size differences exist between weevils from the Manawatu-Wanganui Region compared with other parts of New Zealand.
- Assess whether any size differences are associated with nitrogen and/or phosphorus concentrations in the host plant.
- Provide management advice to improve the performance of the nodding thistle crown weevil in the Manawatu-Wanganui Region.

Methods

- Nodding thistle plants were sourced from seven regional councils: Manawatu-Wanganui, Bay of Plenty, Hawke's Bay, Wellington, Tasman, Canterbury, and Southland. Adult crown weevils were also collected from five of these regions.

Results

- Horizons Regional Council sites had high weevil larval densities, low thistle abundance and smaller thistles compared with other parts of the country.
- There were differences in weevil body size between sites on one property in the Manawatu-Wanganui Region. While early summer-collected weevils at Otupae Station West were mostly similar in size to other parts of the country, weevils on Otupae Station East were heavier, but had smaller elytra (overwings) compared with Otupae West, and compared with weevils from Tasman and Canterbury. Autumn-collected weevils at Otupae West were, again, similar to other parts of the country, but weevils at Otupae East were lighter and/or had smaller elytra compared with Otupae West, Hawke's Bay and Canterbury.
- No clear relationships were identified between nitrogen concentration in thistles and weevil size.
- Phosphorus may be associated to some degree with the smaller size of weevils in autumn at Otupae East.

Conclusions and Recommendations

- Assuming that the Otupae sites are representative of the situation in the Manawatu-Wanganui Region, nodding thistle crown weevils appear to be doing better than in other parts of New Zealand that participated in the survey, contrary to our initial expectation. The reasons why the performance of the weevils in the Manawatu-Wanganui Region is thought to be poorer than other regions remain unclear. A much more detailed and

extensive study would be required to confirm if performance is really worse and the reasons why.

- Efforts should focus on widely establishing the green thistle beetle (*Cassida rubignosa*), which attacks all thistle species, and is likely to reduce nodding thistle populations further.
- There is likely to be some value in continuing to establish the nodding thistle crown weevil at sites in the Manawatu-Wanganui Region where they are not yet present.
- There is no advantage in sourcing weevils for redistribution from outside the Manawatu-Wanganui Region.
- Weevil collection for redistribution should be timed carefully for mid- to late autumn.

1. Introduction

A study to compare the size of a biocontrol agent, nodding thistle crown weevil (*Trichosirocalus horridus*), between different regions of New Zealand, and the relationship with nitrogen and phosphorus levels in the host plant, was undertaken for Horizons Regional Council between July 2009 and April 2010. The aim of the study was to see if the performance of this agent could be improved in the Manawatu-Wanganui Region, where it has been suggested that populations are low and provide inadequate control. This project was funded by an Envirolink medium advice grant (HZLC73).

2. Background

Nodding thistle crown weevil (NTCW), *Trichosirocalus horridus* sensu (Panzer) (Coleoptera: Curculionidae), is an introduced biocontrol agent that contributes both to thistle biomass reduction (short-term) and to depletion of the long-lived seed bank (long-term) of nodding thistle, *Carduus nutans* L. (Asteraceae). In conjunction with other thistle biocontrol agents, the crown weevil has been reported to dramatically reduce nodding thistle infestations in many parts of the country.

In the high country around Taihape and Taumarunui, within the Manawatu-Wanganui Region, populations of NTCW have appeared to be extremely slow to establish, and it has been suggested that populations have tended to remain low, providing inadequate control. The reasons for this poor performance are unclear. Because they have not been able to easily harvest their own weevils, Horizons Regional Council have been sourcing and releasing NTCW from other regions. Recently Horizons' biosecurity staff noticed that weevils sourced from the Hawke's Bay appeared to be substantially smaller than those from Canterbury. This was of interest given that all NTCW present in New Zealand originated from a single source population. Size of biocontrol agents is often a critical factor in their effectiveness, as it can impact on insects' ability to survive winter and withstand extreme weather conditions and extreme weather events. Size of individuals has also been linked to the ability of a species to establish a viable population size, which is a prerequisite to effective weed control.

Variation in the nutritional status of plants (e.g. nitrogen, phosphorus) can have a large impact on the final size of plant-feeding insects such as weed biocontrol agents. The final size of the insect could be detrimental to its ability to survive and, for biocontrol agents, could reduce establishment success. Therefore, there was a possibility that the poor performance of NTCW in the Manawatu-Wanganui Region (and other regions including Waikato where poor performance has been claimed) could be attributed to the size of the weevils there, and a possibility that nutrient levels of the nodding thistle plants could be a limiting factor for weevil growth.

3. Objectives

- Assess whether size differences exist between weevils from the Manawatu-Wanganui Region compared with other parts of New Zealand.
- Assess whether any size differences are associated with nitrogen and/or phosphorus concentrations in the host plant.
- Provide management advice to improve the performance of the nodding thistle crown weevil in the Manawatu-Wanganui Region.

4. Methods

4.1 Thistle collection

Nodding thistle rosettes were collected from two sites at each of the following seven regions: Manawatu-Wanganui, Bay of Plenty, Hawke's Bay, Wellington, Tasman, Canterbury, and Southland.

Participating biosecurity officers were requested to locate two sites with different land use, preferably one dairy (assumed likely to have increased anthropogenic nitrogen) and one other land use, but as similar as possible in any other respect (altitude, weed management). This proved too difficult, and most regions provided both samples from sites with similar land use. Officers were requested to record general information about each site. The collection form is included in Appendix 1.

Rosettes were collected during late winter to early spring. This is the time when weevil larvae are actively feeding and affected by the level of plant nutrients.

The number of NTCW larvae in the rosettes was recorded to get a preliminary measure of population differences between the regions. Any traces of weevil larvae and their frass, which contain much higher concentrations of nutrients than the plant material and could therefore bias the results, were then removed from the rosettes. The clean crowns were dried for at least 24 h at 80˚C to fix nutrient levels until chemical analysis could be done.

4.2 Chemical analysis

Chemical analysis was done at Landcare Research's laboratory in Palmerston North. A Kjeldahl procedure (Smith 1980) was used to determine the percentages of nitrogen and phosphorus per crown dry weight. Nine to 10 crowns per sites were processed (depending on whether the initial amount of material in the sample was sufficient). Samples from three sites were not analysed for the following reasons: Bay of Plenty's Matata site – rosettes collected were winged not nodding thistle; Canterbury's The Mound site – was to be resown by early summer so weevils could not be collected; Wellington's Cape Palliser site – no larvae were recorded from either Wellington site, and no adult weevil collections made, so only Kawakawa Station was subjected to chemical analysis (chosen at random).

4.3 Weevil collection

In early summer, adult weevils were collected from some of the sites used to collect rosettes. It was intended that weevils would be collected from all sites, but at some sites weevils were not found, and some councils that participated in the rosette collection were unable to commit time to adult weevil collection. Altogether, adults were collected from six sites in five regions. Hawke's Bay Regional Council were unable to collect in early summer, before weevils entered summer aestivation, and agreed instead to collect in autumn, when weevils come out from aestivation to begin reproducing. Autumn-collected weevils cannot be directly compared with early-summer-collected ones because autumn weevils are those that have successfully survived a period of aestivation during the summer drought period, without feeding. If larger size is associated with better survival rates, there is a risk that, on average, autumn-collected weevils would be larger than early-summer-collected weevils. Therefore, to be able to make any comparisons between weevils from Hawke's Bay and from the other regions, autumn collection was also requested from two early-summer-collection sites, in the Manawatu-Wanganui and Canterbury regions.

The adult weevils were sexed and oven-dried for dry weight measurements. One elytron (overwing) was plucked from a subsample of a minimum of 50 individuals (25 females and 25 males) per site and scanned. The scanned images were used to determine the length, width, perimeter and area of each elytron, using the computer software WinFolia™. The genitalia of adult males were examined to check that all the weevils sit within the same species (for more details about the species complex and identification difficulty see Alonso-Zarazaga & Sánchez-Ruiz (2002) and Groenteman et al. (2008)).

4.4 Statistical analysis

Weevil dry weight and elytron area (representing elytron size) were both subjected to analysis of variance (ANOVA), with sex (females vs males) and site and their interaction as explanatory variables. These tests were conducted separately for early-summer- and autumn-collected weevils. Nutrient contents of nodding thistle rosettes were also subjected to ANOVA, with site as the explanatory variable. Sex ratios were subjected to a binomial test. Analyses were done using the statistical package R (R Development Core Team 2010).

5. Results

5.1 Geographic distribution of the survey

Regional councils that participated were (from north to south): Bay of Plenty, Hawke's Bay, Manawatu-Wanganui, Wellington, Tasman, Canterbury and Southland (Fig. 1). General information about each site is summarised in Table 1.

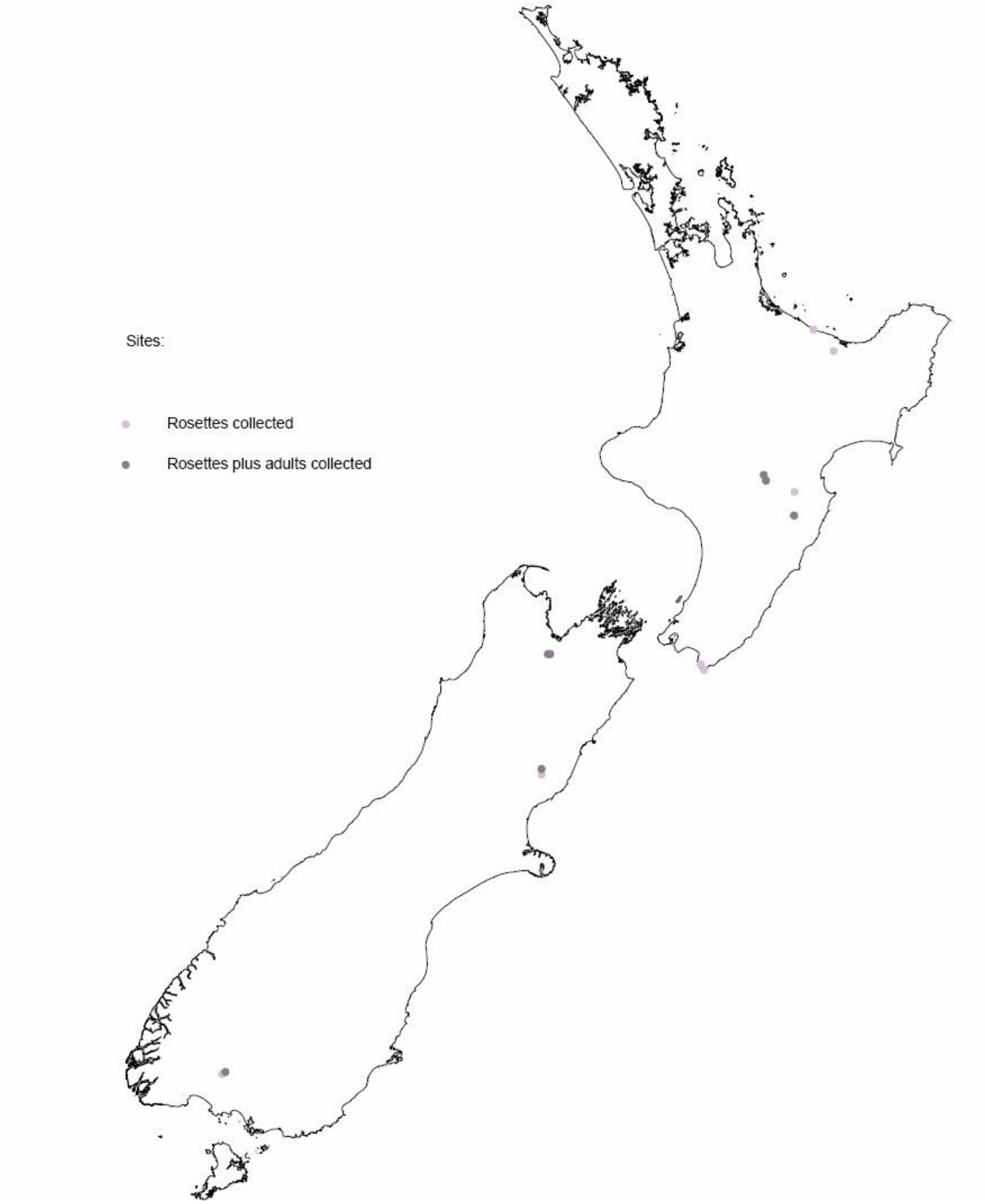


Figure 1 Distribution of nodding thistle rosettes and crown weevil collection sites.

Table 1 Site information. All sites provided rosettes. Adult weevils were collected from the sites in bold

Region	Site	Thistle abundance	Land use	Other means for nodding thistle control	Changes in nodding thistle over time
Canterbury	The Mound Rd	Moderately common	Dairy	None	No comment made
Canterbury	**Leaches Rd**	Very common	Sheep & beef	None	Only became a problem 20 years ago. Now declining.
Bay of Plenty	Sisams	No comment made	Sheep & beef	None	No comment made
Bay of Plenty	Matata	No comment made	Sheep & beef	None	Big reduction
Hawke's Bay	**Brow Linburn Rd**	Moderately common	Beef	Unknown	Slightly declining
Hawke's Bay	Limeworks block - Tikokino Rd	Moderately common	Sheep & beef	Spraying sometimes	Ups and downs with droughts and regrassing of paddock
Manawatu-Wanganui	**Otupae Station East**	Rare	Sheep & beef	None	No difference noticed
Manawatu-Wanganui	**Otupae Station West**	Moderately common	Sheep & beef	None	No
Greater Wellington	Kawakawa Station	Moderately common	Sheep & beef	None	Declined
Greater Wellington	Cape Palliser	Moderately common	Sheep & beef	None	No
Southland	**Gorge Rd Quarry**	Moderately common	Quarry & sheep/ beef	None	Appear stable. Plants on steep cliff keep contributing seeds
Southland	Birchwood Rd	No comment made	Sheep & beef	Spray and heavy stocking	Increased
Tasman	**Dicks**	No comment made	Beef & goats	Spray bare ground	Declined since land use change (two years ago from sheep & beef)
Tasman	**Wantwood**	No comment made	Sheep & beef	None	Decline

5.2 Size of adult weevils

All morphological parameters taken (elytron length, width, perimeter and area) displayed a similar pattern. Therefore only one parameter, elytron area, was selected to represent the morphological aspect. In addition, dry weight is presented.

Seasonal differences

For weevils from Manawatu-Wanganui, elytron area of early-summer-collected weevils was significantly larger than of autumn-collected ones (Table 2). There was no significant difference in dry weight between seasons.

Table 2 ANOVA for the effects of sex (males vs females), collection season (early summer vs autumn) and site (East vs West Otupae Station) on adult weevil dry weight and elytron area. Significant differences are in bold

	Dry weight (mg)				Elytron area (mm^2)			
	df	Sum of Sq	*F*	Pr(>*F*)	df	Sum of Sq	*F*	Pr(>*F*)
Sex	1	20.61	7.85	**0.005**	1	4.08	51.57	**<0.001**
Season	1	1.1	0.42	0.518	1	0.91	11.48	**0.001**
Site	1	0.73	0.28	0.598	1	0.49	6.19	**0.014**
Interaction: sex × season	1	2.08	0.79	0.374	1	0.21	2.70	0.102
Interaction: sex × site	1	0.32	0.12	0.726	1	0.02	0.22	0.642
Interaction: season × site	1	7.00	2.66	0.104	1	0.13	1.69	0.195
Interaction: sex × season × site	1	1.28	0.49	0.485	1	0.01	0.19	0.665
Residuals	253	664.58			173	13.70		

Site and sex differences

Weevils from Otupae Station West (Manawatu-Wanganui) had significantly *larger* elytra (but were *not heavier*) than weevils from Otupae East. Females were consistently *heavier* and had *larger* elytra than males (Table 2).

The seasonal differences dictated that data from summer- and autumn-collected weevils had to be analysed separately for the datasets including multiple regions.

Dry weight

In early summer, weevils collected at Manawatu-Wanganui's Otupae Station East were significantly *heavier* than weevils collected from Tasman (Dicks), and marginally non-significantly heavier than weevils from Otupae West and weevils collected from Canterbury (Fig. 2, Table 3). In autumn, weevils collected at Otupae East were significantly *lighter* than weevils collected from the Otupae West and from Hawke's Bay weevils (Fig. 2, Table 3).

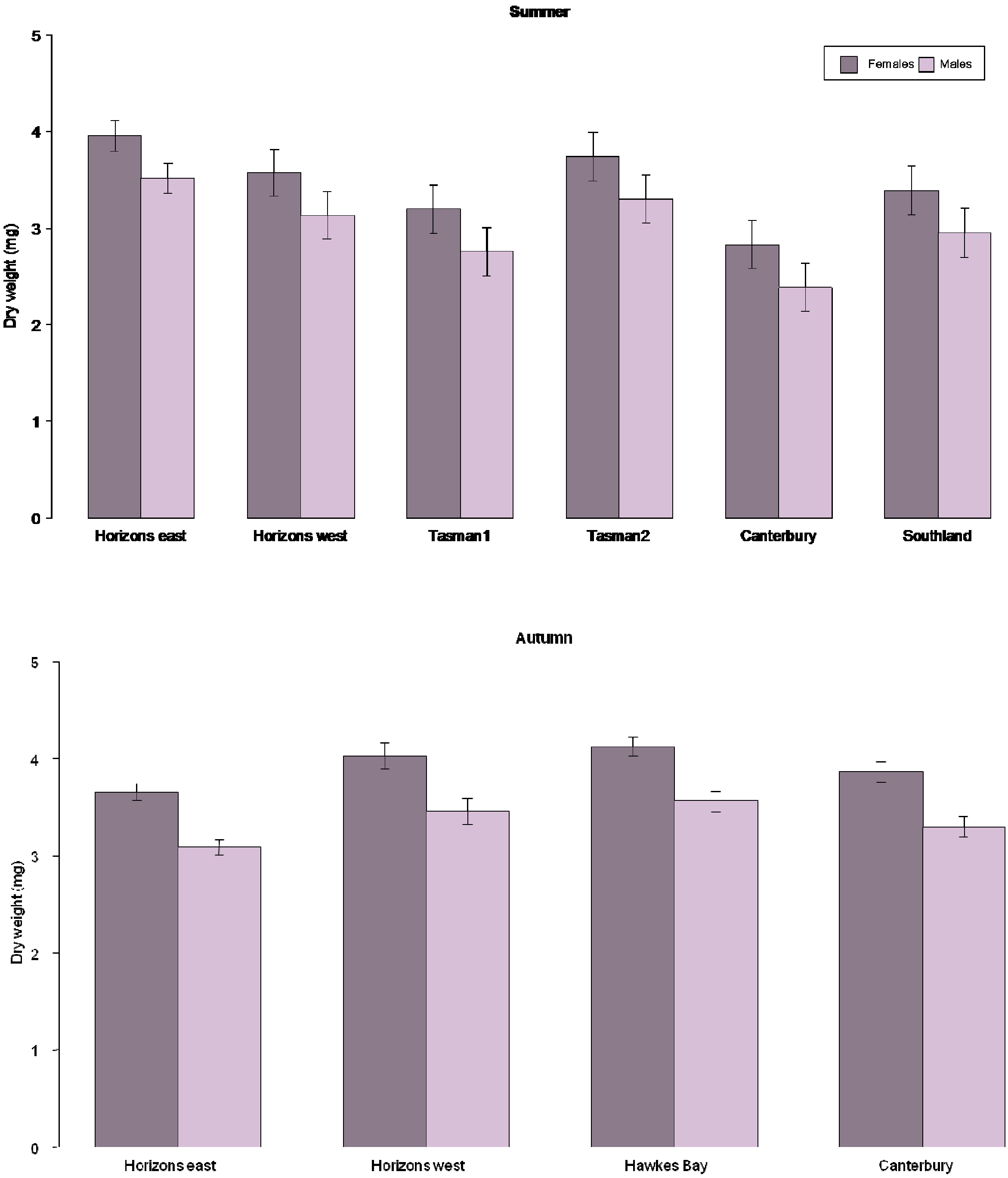


Figure 2 Regional differences in weevil dry weight (back-transformed means ± SE).

Table 3 ANOVA for the effects of site, sex (males vs females), and their interaction on differences in weevil dry weight. Significant differences are in bold

	Early summer				Autumn			
	df	Mean Sq	*F*	Pr(>*F*)	df	Mean Sq	*F*	Pr(>*F*)
Site	5	10.65	4.79	**<0.001**	3	4.227	11.706	**<0.001**
Sex	1	17.56	7.89	**0.005**	1	16.157	44.743	**<0.001**
Interaction	5	0.92	0.41	0.839	3	0.244	0.675	0.568
Residuals	351	2.23			210	0.361		

Elytron area

In early summer, the elytron area of weevils collected in both the eastern and western sides of Manawatu-Wanganui's Otupae Station was significantly smaller than that of weevils collected from the two Tasman sites, Canterbury and Southland (Table 4, Fig. 3). In autumn, the elytron area of weevils collected at Otupae East was significantly smaller than that of weevils collected from Otupae West and from Hawke's Bay and Canterbury (Table 4, Fig. 3).

Table 4 ANOVA for the effects of site, sex (males vs females), and their interaction on differences in elytron area. Significant differences are in bold

	Early summer				Autumn			
	df	Mean Sq	*F*	Pr(>*F*)	df	Mean Sq	*F*	Pr(>*F*)
Site	5	0.59	7.47	**<0.001**	3	0.50	5.72	**0.001**
Sex	1	7.78	97.87	**<0.001**	1	6.16	70.52	**<0.001**
Interaction	5	0.09	1.13	0.346	3	0.03	0.34	0.799
Residuals	292	0.08			175	0.09		

5.3 Rosette size and larval counts

The two sites on Manawatu-Wanganui's Otupae Station had a high proportion of small rosettes, a low to medium proportion of medium-sized rosettes, and no large rosettes at all; in addition, these sites exhibited high densities of larvae per rosette in comparison with most other regions (Fig. 4), and low thistle abundance (Table 1; subjective assessment). It is interesting to note that nodding thistle can exhibit different life strategies, which are associated with size: if conditions are favourable and there are no stress factors such as drought and/or herbivory, nodding thistle may remain at the rosette stage for two years, increasing in size before flowering and terminating its life. However, when stressed, nodding thistle could flower in its first year of life, even if it only reached a small size. Therefore, if nodding thistle populations are constantly under herbivory pressure they are likely to consist of mainly annual individuals, which tend to be smaller (i.e. less successful).

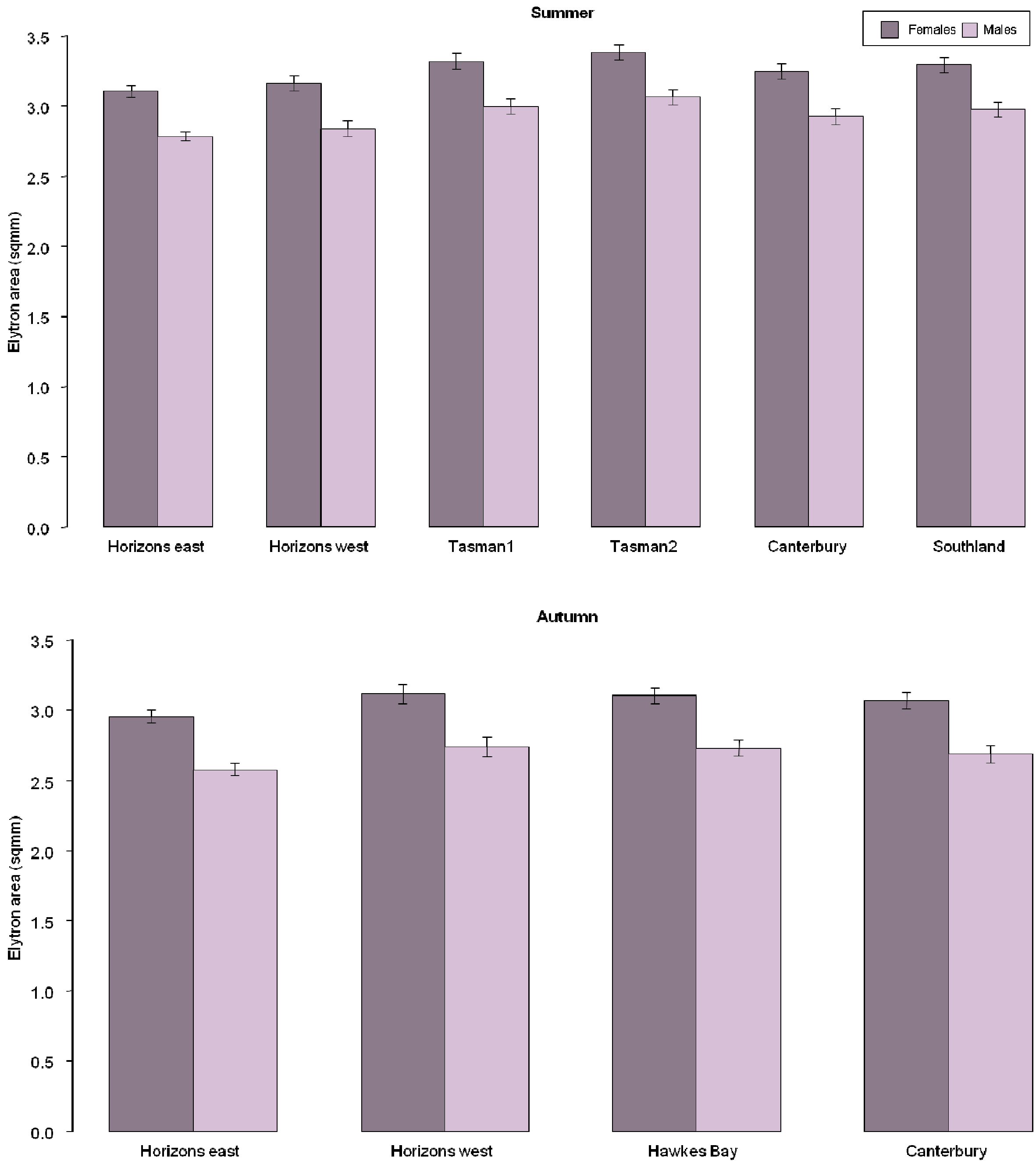


Figure 3 Regional differences in elytron area (back-transformed means ± SE).

It is possible that the Manawatu-Wanganui thistle populations, with generally small rosettes and high larval densities, represent this state; however, it is difficult to say this with any certainty from a single snapshot sampling event, because of the biennial nature of nodding thistle. Still, some supporting evidence could be gained from the pattern observed in the other regions sampled in this survey: regions like Canterbury and Tasman with relatively high larval counts also have rosette size distributions tending towards the smaller sizes, whereas regions in which no larvae were recorded, such as Bay of Plenty and Wellington, have the greatest proportion of large rosettes (Fig. 4). While only a snapshot, the wide geographic scale of this pattern provides strong circumstantial evidence to support NTCW contribution to nodding thistle control. It has to be noted that thistle density was not quantified in this survey. The officers were requested to make a subjective comment about the density of infestation (thistles are rare/moderately common/very common), but not all forms returned had a density category selected.

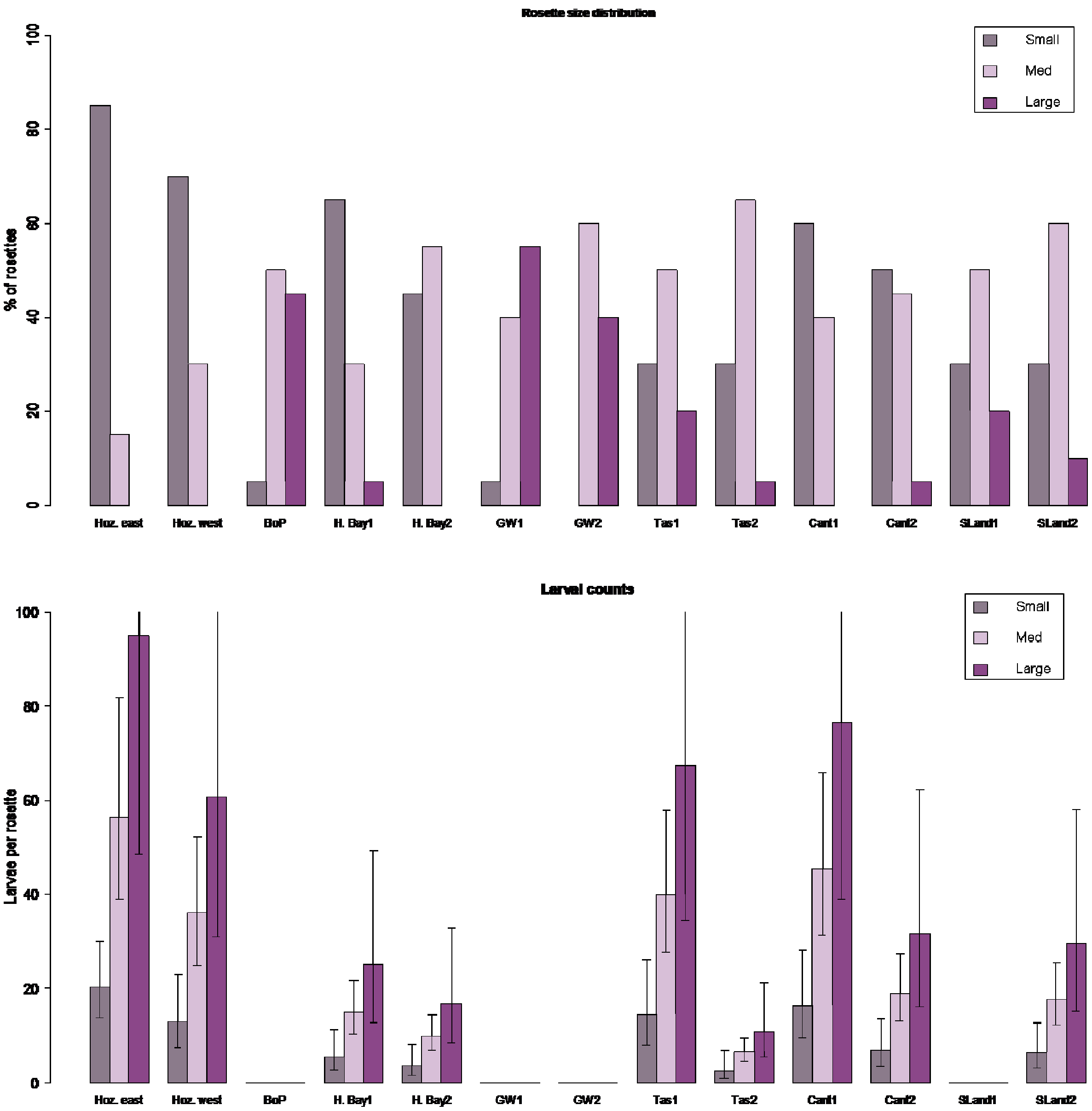
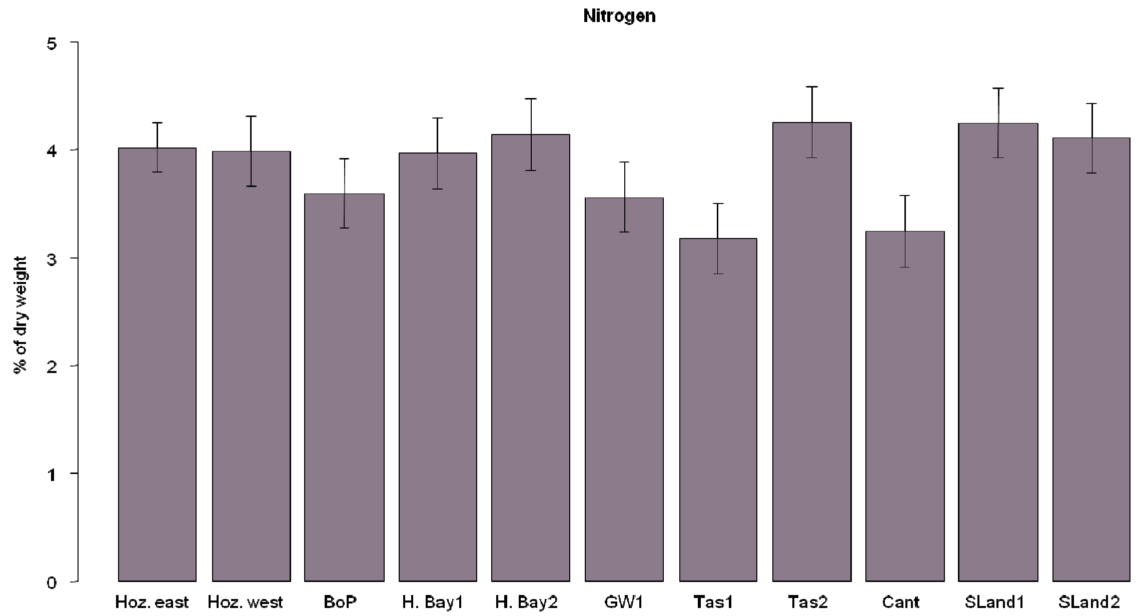


Figure 4 Rosette size distribution (percentages) and the corresponding number of larvae per rosette in each rosette size category (back-transformed mean ± 95% CI) from two sites per region (only one site shown for Bay of Plenty; rosettes from the second site were winged thistle).

The codes for site names are (from left to right): Hoz. East and Hoz. West = Horizons, Otupae Station, east and west sides respectively; BoP = Bay of Plenty, Sisams; H. Bay1 and H. Bay2 = Hawke's Bay Brow Linburn and Limestone works sites respectively; GW1 and GW2 = Greater Wellington Kawakawa and Cape Palliser sites respectively; Tas1 and Tas2 = Tasman Dicks and Wantwood sites respectively; Cant1 and Cant2 = Canterbury Leaches and The Mound sites respectively; SLand 1 and SLand 2 = Southland Quarry and Birchwood sites respectively.

5.4 Nutrients

Nitrogen levels at both sites at Manawatu-Wanganui's Otupae Station were not significantly different from most other sites, and were significantly higher than the level at Tasman's Dicks site and Canterbury's Leaches site (Fig. 5). Phosphorus level at Otupae East was significantly lower than at Otupae West, as well as all other sites except Canterbury's Leaches site. Phosphorus level at the latter was similar to that at Otupae East (Fig. 5). Phosphorus levels at Hawke's Bay's Limeworks site, at Tasmans' Wantwood site and at Southland's Birchwood site were significantly higher than at all other sites.



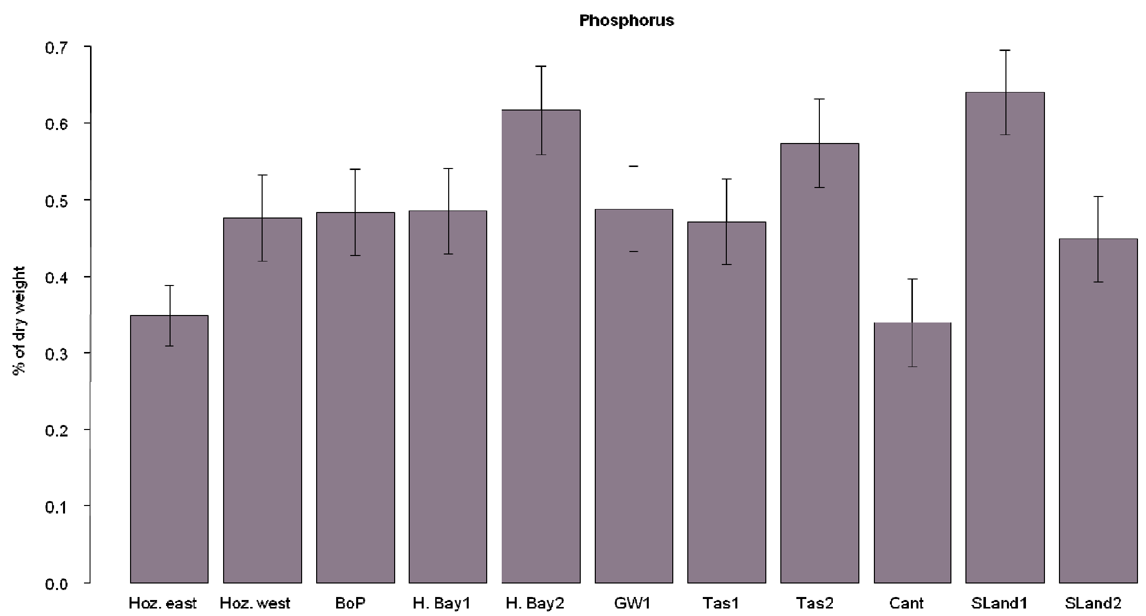


Figure 5 Nitrogen and phosphorus as percentage of dry weight of nodding thistle rosettes across sites (back-transformed means ± SE). For site name codes see Fig. 4 caption.

Nutrient concentrations generally did not correspond well with size patterns. The few patterns that could be detected are summarised in Table 5. From Table 5 it could be inferred that lighter dry weight and smaller elytra at Otupae East compared with Hawke's Bay and Otupae West weevils may be associated with lower levels of phosphorus. However, phosphorus levels at Otupae East were similar to those at Canterbury, so deficiency of this nutrient cannot explain the difference in elytron area between these two sites. Higher nitrogen levels at Otupae East compared with Tasman and Canterbury may explain the heavier dry weight of weevils there in early summer, but cannot explain the difference from Otupae West. It also cannot explain the smaller elytron area at Otupae East compared with Tasman and Canterbury sites.

Both higher nitrogen and higher phosphorus corresponded with heavier dry weight at the Tasman, Canterbury and Southland sites. However, the sites with lower nutrient concentrations also had higher larval densities, so smaller adult size in these cases may also be associated with competition within the crown at the larval stages. Elytron area did not demonstrate a similar pattern to dry weight in those sites.

Table 5 Patterns showing some degree of association between nitrogen and phosphorus levels at Manawatu-Wanganui's East Otupae Station and weevil size parameters

Otupae East:		Early summer				Autumn		
Dry weight	>	Tasman	Canterbury	Otupae West	<	Otupae West	Hawke's Bay	
Elytron area	<	Tasman	Canterbury	Southland	<	Otupae West	Hawke's Bay	Canterbury
Nitrogen	>	Tasman	Canterbury		>			Canterbury
Phosphorus	≅		Canterbury		<	Otupae West	Hawke's Bay	

6. Conclusions

This study has not been able to explain why anecdotally NTCW is believed to have performed more poorly in the Manawatu-Wanganui Region than other regions, and has provided some evidence to the contrary. Assuming that Otupae Station sites are representative of NTCW sites in the Manawatu-Wanganui Region, nodding thistles in this region seem to support NTCW populations that are no smaller in numbers than in the other parts of New Zealand that participated in the survey: larval density at the Manawatu-Wanganui sites was at the higher end of the scale, and both sites supported a high population of adults in early summer. The only body size difference emerged in autumn. Lighter dry weight in autumn is not surprising, as weevils emerge from a long period without feeding over summer. Smaller elytron area in autumn is more difficult to explain, because it could be expected that larger individuals will better survive the summer aestivation period. One possible explanation is that smaller weevils emerge from aestivation sooner than large weevils because they run out of fat reserves sooner. Supporting evidence comes from the heather beetle, *Lochmaea suturalis*, in the central North Island: smaller beetles emerge from overwintering earlier than do larger beetles, and fat reserves in beetles emerging early are lower than in beetles that maintain longer overwintering periods (P. Peterson, pers. comm.). Collecting NTCW in early autumn in a dry year, before aestivation could be expected to have fully finished for all weevils, could have meant sampling was biased in favour of small weevils. Another possible explanation may be that larger individuals may have better dispersal ability and may be more likely to migrate. Finally, a further possible explanation may relate to the role of other thistle species available at each site as alternative hosts; however, presence of other thistle species was not recorded in this study.

There was some evidence that nitrogen may be correlated with weevil dry weight, but not with morphological size (represented by elytron area). It was impossible to separate the pattern of dry weight differences from larval density (and hence potential competition). Lower phosphorus was to some degree correlated with smaller weevils.

The study presented here was designed to detect clear patterns if they occurred. Any complicated patterns were never intended to be possible to interpret due to the following limitation of the system: (1) The study only covered a small number of sites, and they were not selected at random; (2) The study only covered one year, while nodding thistle can be biennial, and is highly sensitive to different farm management operations; (3) While sampling protocols were provided, it was impossible to guarantee that they were strictly and similarly followed by the different samplers. Hence, a much more detailed and extensive study would be required to confirm if performance of NTCW is really worse in the Manawatu-Wanganui region than in other regions and the reasons why. Given the resources required for such a study and the fact that new thistle agents like the green thistle beetle (*Cassida rubignosa*) are now available that are likely to improve thistle control, resources would probably be better directed at the latter.

7. Recommendations

- Efforts should focus on widely establishing the green thistle beetle (*Cassida rubignosa*), which attacks all thistle species, and is likely to reduce nodding thistle populations further.
- There is likely to be some value in continuing to establish the nodding thistle crown weevil at sites in the Manawatu-Wanganui Region where they are not yet present.
- There is no advantage in sourcing weevils for redistribution from outside the Manawatu-Wanganui Region.
- Weevil collection for redistribution should be timed carefully for mid- to late autumn to allow weevils sufficient time to emerge from summer aestivation.
- Phosphorus enrichment on a small scale may increase the size of weevils, but this would need to be tested.

8. Acknowledgements

This survey could not have taken place without the help of regional council staff who collected rosettes and adult weevils at different times of the year: Des Pooley (Bay of Plenty), Darin Underhill (Hawke's Bay), Harvey Phillips (Greater Wellington), Robin Van Zoelen and Lindsay Grueber (Tasman), Jesse Bythell and Peter Ayson (Southland), and finally, Malinda Matthewson and Don Clark (Horizons), who spotted the size difference and initiated this project. I also thank Tom Dutton for the chemical analysis, Aimee McKinnon, Alex Mathieu and Emilie Robin for technical assistance, and Quentin Paynter for reviewing an earlier draft of the report. This project was funded by an Envirolink grant (HZLC73).

9. References

Alonso-Zarazaga MA, 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.

Groenteman R, Kelly D, Fowler SV, Bourdôt GW 2008. Which species of the thistle biocontrol agent Trichosirocalus are present in New Zealand? In: Julien MH, Sforza R, Bon MC, Evans HC, Hatcher PE, Hinz HL, Rector BG eds XII International Symposium on Biological Control of Weeds, La Grande Motte, France, 22–27 April 2007. Pp. 145–149.

R Development Core Team 2010. R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Smith VR 1980. A phenol-hypochlorite manual determination of amonium-nitrogen in Kjeldahl digests of plant tissue. Communications in Soil Science and Plant Analysis 11: 709–722.

Appendix 1 Nodding thistle crown sampling sheet

Name of collector & contact details Collection date	
Site collected from (site name, region, GPS, altitude)	
Is nodding thistle (circle):	Rare/moderately common/very common Evenly spread/patchy
What is the most common rosette size category based on rough estimate of longest leaf length? (circle)	Smaller than 10 cm 10 to 30 cm Larger than 30 cm A relatively even mix
Was crown weevil released at this site? When? If not when was it first noticed here?	
Are other nodding thistle agents established (circle)?	Receptacle weevil: Yes/No/Unsure Gall fly: Yes/No/Unsure
What other control methods against nodding thistle are used on this site?	
Land use (circle)	Sheep Beef Dairy Deer Other (please specify):
Has land use changed in recent years? When? What was the previous use?	
Has the current owner owned the property long? Can they comment on whether nodding thistle has declined/increased?	

Other comments: