Clover root weevil tolerance of clover cultivars

C.M. FERGUSON, D.M. BARTON and B.A. PHILIP
AgResearch Ltd, Invermay Agriculture Centre, Private Bag 50034, Mosgiel, New Zealand
colin.ferguson@agresearch.co.nz

Abstract
Clover root weevil arrived in New Zealand about 20 years ago causing major loss of productivity as it progressively spread across the whole country. It is now largely controlled by an introduced parasitic wasp biocontrol agent *Microctonus aethiopoides* (Irish ecotype). However, management of insect pests should not rely on a single mechanism and clovers resistant or tolerant to this weevil would be a useful augmentation for farmers to have. This investigation reports on the suitability of 22 clover cultivars to attack from the weevil. Results have shown that contrary to popular belief, red clovers are not universally less favourable to the weevil than white clovers and usefully, within both species cultivar differences point to the possibility of resistance to this pest.

Keywords: Clover root weevil, *Sitona obseletus*, clover cultivars

Introduction
Clover root weevil (CRW) (*Sitona obsoletus* (Gmelin, 1790)) (formerly *S. lepidus*) was first detected in New Zealand in 1996 (Barratt *et al.* 1996) although it was present in Waikato pastures at least a year earlier (Barker *et al*. 1996; Eerens *et al*. 2005). By 2006 it was throughout the North Island and by 2015 throughout the South Island. Adult weevils feed on clover foliage and larval stages on roots and root nodules reducing nitrogen fixation, clover and pasture production, and in severe cases killing clover plants. It was rapidly assigned major pest status (Eerens *et al.* 2005) despite only being a minor pest in its natural range. New Zealand pastures offered an extensive and abundant food source, lack of competition and a scarcity of natural enemies although the latter was addressed by the 2006 release in of the biocontrol agent *Microctonus aethiopoides* Loan (Irish ecotype).

Adult weevils generally emerge from pasture soils in late spring and again in autumn (Gerard *et al*.1999) when they feed on clover foliage particularly favouring newly germinated seedlings (Hardwick & Harens 2000). The characteristic crescent-shaped notches in the leaves caused by adult feeding are the most obvious sign of infestation and, when weevils are abundant, such defoliation can be noticeable. However, while this activity causes loss of photosynthetic area, white clover is adapted for frequent defoliation and direct production loss by CRW adult feeding is small. For example, Gerard & Hackell (2005) estimated a 50 kg DM /ha/year clover loss for a typical Waikato population whereas total clover production varies from 1-7 t DM/ha/year (Anon 2016). Adult weevils live for several months and females lay several hundred eggs which are scattered on the pasture surface where they hatch. It is the ensuing soil-dwelling larvae that are the most damaging. The newly hatched larvae burrow into the soil, locate and feed on root nodules, then as they mature, they move on to the lateral roots, nodal roots and stolons (Gerard 2001; Gerard *et al.* 2004). Larvae are present throughout the year putting continual pressure on clover roots. Root nodule destruction reduces the nitrogen fixing ability of clover. When sufficient larvae are present the pressure on the root system can be so great that clover plants struggle to survive especially when other stresses such as grazing, low soil fertility and adverse climatic events also put the plants under pressure.

The introduction of *M. aethiopoides* for the biocontrol of CRW has been spectacularly successful. However, biocontrol should be considered only one component of pest management and should be augmented with other approaches. Selection of clover cultivars that are tolerant of or resistant to CRW feeding are another means of managing this pest. CRW feeds almost exclusively on clovers (*Trifolium* sp.) and will only attempt to feed on other plants when no alternative is available (Murray 1996a; Hardwick 1998). Within *Trifolium* spp. it feeds on white clover (*T. repens*), red clover (*T. pratense*), strawberry clover (*T. fragiferum*), alsike clover (*T. hybridum*), caucasian clover (*T. ambiguum*), crimson clover (*T. incarnatum*), subterranean clover (*T. subterraneum*), suckling clover (*T. dubium*), clustered clover (*T. glomeratum*) and striated clover (*T. striata*) (Murray 1996a; Hardwick 1998; Crush *et al.* 2007, 2008).

Of the two most common New Zealand pasture clover species, CRW is generally considered, from laboratory studies, to prefer white over red (e.g. Murray 1996a; Gerard & Crush 2003), but these studies are not consistent and Murray & Clements (1994) found no evidence for this. Observations in New Zealand pastures have also suggested that white is the preferred type (Eerens *et al.* 2001; Gerard & Crush 2003) but the latter also reported that CRW appeared able to habituate to red clover. Preferences by CRW adults for some white

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online)

clover cultivars over others have been reported (Murray 1996b; Crush *et al*. 2010) and, in field investigations, some lines of both white and red have shown increased tolerance of CRW feeding relative to others (Eerens *et al*. 2001; Cooper *et al*. 2003). Gerard *et al*. (2005) also detected some resistance within two selections of red clover resulting from levels of formononetin that increased in response to CRW adult feeding.

This investigation took advantage of an already-established agronomic clover cultivar trial that came under attack from CRW. The objectives were to determine whether CRW populations were affected by clover cultivars or species and to compare winter larval survival under different cultivars and species.

Methods

The trial used was established in 2012 by DLF Seeds and was located approximately 9 km south east of Gore, near Kaiwera, in Southland. Fourteen cultivars of white clover and eight of red (Table 1) were sown as pure stands in 1.5 x 10 m plots. The clover cultivar plots were placed in four randomised blocks.

Adult CRW

Autumn adult CRW abundance was assessed in each plot on 14 May 2014 by sampling by suction 4 x 10 m strips each 0.12 m wide, using a Stihl Blower/Vacuum/ Shredder SH56C (suction = 710 m^3 air/h) with a mesh bag fitted to the vacuum intake. CRW were extracted from the collected litter using modified Berlese heat extraction funnels. In October 2014, 51 adult CRW were collected from the trial site and dissected to determine if the biocontrol agent was present.

Larval CRW

Twenty 2.5 cm dia. x 7.5 cm deep soil cores were sampled from each plot on 14 May 2014 and 40 more cores per plot on were sampled 16 October to estimate autumn and spring larval densities. Each core was taken from the base of a plant avoiding plot edges. The larvae were extracted by hand crumbling the soil until a fine tilth.

Data analyses

For each data set, adults, larvae sampled in May and larvae sampled in October the counts were log transformed for Genstat analyses of variance. In each case comparisons between mean densities for the cultivars were carried out using Tukey least significant differences (5%). For ease of interpretation back transformed means (with larval numbers converted to number/m^2) are presented in Table 1, and Figures 1 and 2.

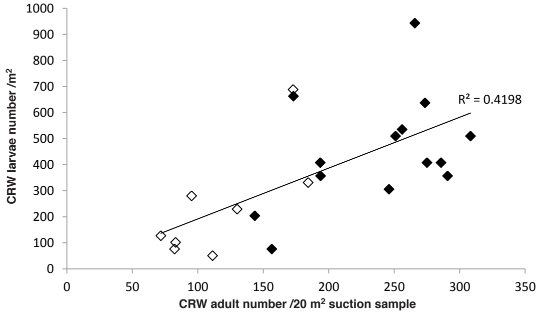

Figure 1 Larval and adult numbers of CRW in May 2014. Open and solid symbols represent red and white clover cultivars, respectively.

Results and Discussion

Adult CRW

Because the plots were narrow and without buffer zones it is likely that the CRW adults could easily move between them should there be incentive to do so. However, the weevil populations throughout the trial site had likely been resident for several years and new adults emerging from the soil in spring and autumn would have had ample time to locate habitats they found suitable and avoid habitats that were not. Therefore it is reasonable to assume the populations were “settled” and not inhabiting plots where the clover variety was unfavourable. The relationship between adult and larval numbers (Figure 1) in which low adult numbers are generally associated with low larval numbers suggests that there was some within-cultivar stability.

Adult CRW were collected from all clover cultivar plots in reasonable numbers (>70) (Table 1). Overall significantly more were collected from white clover than red clover plots ($P<0.001$), however, results also indicated that red clover is far from being an unfavourable host plant. Within red clover cultivars, significantly more CRW were collected from cv. Rajah and cv. Rossi than from cv.Vesna and the numbers in Rajah and Rossi were well within the range collected in white clover cultivars. Within the white clover, significantly ($P<0.05$) fewer CRW were collected from cv. Quest than from the nine other white clover cultivars, but none were significantly different from the next lowest cv. Rivendel (Table 1).

Microctonus aethiopoides was not found at the trial site. This does not preclude its presence but does indicate that it was unlikely to be influencing the sampled CRW populations.

Larval CRW

Core sampling for larvae was a quantitative measure of CRW density but there could have been underestimation as many small larvae may have been overlooked. However, the larvae found were predominantly 3rd instar and older, and younger larvae were considered uncommon at the sampling times chosen. The larval densities measured were similar to those found elsewhere in New Zealand (e.g. Cooper *et al*. 2003) before the biocontrol release for both red and white clover cultivars. This indicates that CRW were as important in the southern South Island as they were further north and that the southern climate, as could be expected given CRW's Palearctic native range, was no constraint to population build up.

As with adult CRW, significant differences were detected in mean larval densities between red and white clover both in May (P=0.021) and October (P<0.001) (Table 1) and again white clover supported more larvae, overall, than did red clover on both occasions. Notably, however, there were overlaps; some red cultivars supported as many, or more, larvae than some white cultivars (Table 1, Figure 2). This was

Table 1 Mean numbers of CRW adults (back transformed means) collected from clover cultivars in May and estimated densities of larvae (Nº/m^2) in May and October 2014.

		Adults	Larvae, May	Larvae, October
Red cultivars	Suez	107	144 [b]	157
	Tuscan	78	144 [b]	105
	Astred	80	172	153
	Vesna	71 [b]	210	95
	Lone	120	288	174
	Sensation	91	323	149
	Rajah	181 [a]	413	212
	Rossi	164 [a]	751 [a]	105
White cultivars	Rivendel	137	160 [c]	327
	Quest	100 [c]	264	452
	Tahora II	288 [d]	355	235
	Tribute	170	363	374
	Aran	240 [d]	380	314
	Kotare	262 [d]	431	390
	Apex	271 [d]	433	526 [d]
	Klondike	193	467	256
	Mainstay	297 [d]	528	119 [c]
	Kopu II	251 [d]	570	203
	Weka	244 [d]	575	194
	Bounty	263 [d]	699 [d]	507
	Demand	258 [d]	745 [d]	467
	Riesling	173	756 [d]	436
Species	Red	106 [e]	261 [e]	139 [e]
	White	215 [f]	444 [f]	318 [f]

Cultivars and species in the same column marked with [a] are significantly different to [b], [c] to [d] and [e] to [f].

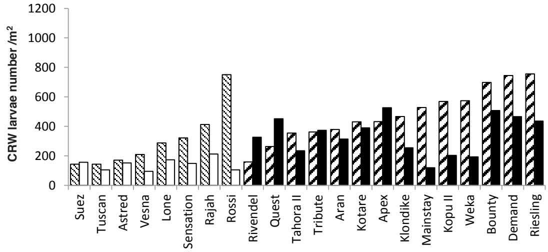

Figure 2 Larval densities (number/m^2) (back transformed means) of CRW in May and October 2014. Lightly patterned bars represent red clover cultivars sampled in May, clear bars red clover cultivars sampled in October, heavily patterned bars white clover cultivars sampled in May and black bars white clover cultivars sampled in October.

more pronounced in May than October but indicates that a general statement of white clover being more favourable than red clover, based predominantly on adult feeding assays, is misleading. This investigation did not examine if CRW larvae perform less well on red clover than white (Crush *et al*. 2010) and this should be further investigated particularly given reported habituation to red clover (Gerard & Crush 2003).

The results show clover species affected both above and below ground CRW populations. Within the red cultivars in May, cv. Rossi supported significantly more larvae than either Suez or Tuscan (Table 1) but in October no significant differences were detected. Interestingly, however, the decrease in larvae under Rossi from May to October was marked (91%) and significant (P<0.05) (Table 1), warranting further investigation.

Within white clover cultivars Rivendel supported fewer larvae than Bounty, Demand and Riesling in May (Table 1) but by October these differences were not apparent. Between May and October Mainstay exhibited a large decrease in larval numbers (Table 1, Figure 2) and in October this was significantly less than Apex which supported the most CRW. Similar, but non-significant changes were also found for Kopu II and Weka (Table 1, Figure 2). As for Rossi, these apparent reductions need further evaluation as any such decline in larval numbers over winter will result in reduced pressure on clover plants and give rise to fewer adults in early summer. Crush *et al* (2005) indicated some success in developing clover lines with tolerance of CRW although generally these were not commercial cultivars. An understanding of larval decline under the different cultivars in this investigation may provide other avenues to pursue in combating CRW. Other factors not assessed in this investigation may have influenced the larval densities detected and differences between cultivars. The availability of root nodules is known to influence larval establishment with adult weevils able to detect plants with nodules (Johnson *et al*. 2006) and preferentially lay eggs on those rather than plants that have not formed nodules. As soil nitrogen levels increase with time in pure clover swards some cultivars may not have had as much need to produce or replace nodules as within a mixed sward and if so reduced levels of nodules may have reduced weevil oviposition in some plots. Depth of nodule formation in the soil may affect larval survival as the small newly hatched larvae must burrow in the soil to locate them. If the nodules are deep in the soil the larvae may be unable to reach them. Nodule size may also play a role in larval densities as few large nodules may support fewer larvae than greater numbers of smaller nodules.

Conclusion

CRW appears to favour some clover cultivars over others within species and while overall red clover supports lower populations than white there was considerable overlap. The results indicated preference for some cultivars over others and do not address CRW impact if the weevils had no choice of food plant. They do, however, indicate that even the least preferred cultivars tested here support what are generally considered to be moderate CRW densities that will result in reductions in the amount of nitrogen fixed by the plants, loss of roots and allow ingress of root pathogens via feeding lesions. This does not negate the potential benefits that would result from increased levels of either clover resistance or tolerance, both of which would be complementary to biocontrol. The investigation showed that some cultivars supported lower CRW levels, especially after winter. While the mechanisms associated with differing CRW levels remain unknown, the differences suggest there are both above- and below-ground cultivar attributes that could be exploited to develop more pest resistant or tolerant clovers and this warrants further investigation.

ACKNOWLEDGEMENTS

The authors thank Beef + Lamb New Zealand for funding this investigation (FITT Application 14FT03). They also thank Robert and Stacey Young as project leaders and for access to the trial site, and Gavin Milne (DLF Seeds) for allowing the trial to be used in this investigation and facilitating the project.

Rachael Macdonald provided technical support with CRW sampling and data collection. Statistical analyses of the data were carried out by Dr Neil Cox, Bioinformatics Maths & Statistics, AgResearch Ltd, Invermay Agricultural Centre.

REFERENCES

Anon. 2016. Clover. www.dairynz.co.nz/feed/pasture-renewal/select-pasture-species/clover/. Accessed 21 July 2016.

Barker, G.M.; Addison, P.J.; Firth, A.C.; Barratt, B.I.P. 1996. *Sitona lepidus* Gyllenhal newly established in New Zealand: Assessment of distribution in the North Island. *New Zealand Plant Protection 49*: 266-269.

Barratt, B.I.P.; Barker, G.M.; Addison, P.J. 1996. *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), a potential clover pest new to New Zealand. *New Zealand Entomologist 19*: 23-30.

Cooper, B.M.; Crush, J.R.; Woodfield, D.R.; Willoughby, B.E.; Pankhurst, K.A. 2003. Red clover legume showing tolerance to clover root weevil. *Proceedings of the New Zealand Grassland Association 65*: 153-156.

Crush, J.R.; Cooper, B.M.; Woodfield, D.R.; Gerard, P.J. 2005. Development of clovers that are tolerant to clover root weevil. *Proceedings of the New Zealand Grassland Association 67*: 23-27.

Crush, J.R.; Ouyang, L.; Gerard, P.J.; Rasmussen, S. 2007. Effect of clover root weevil on four annual forage legumes. *Proceedings of the New Zealand Grassland Association 69*: 213-217.

Crush, J.R.; Gerard, P.J.; Ouyang, L.; Wilson, D.J. 2008. Effects of clover root weevil and moisture stress on subterranean, balansa, Persian, arrowleaf and white clovers. *Proceedings of the New Zealand Grassland Association 70*: 255-257.

Crush, J.R.; Gerard, P.J.; Ouyang, L.; Cooper, B.M.; Cousins, G. 2010. Effect of clover root weevil larval feeding on growth of clover progenies from parents selected for tolerance in field trials. *New Zealand Journal of Agricultural Research 53*: 227-234.

Eerens, J.P.J.; Cooper, B.M.; Willoughby, B.E.; Woodfield, D.R. 2001. Searching for clover root weevil (*Sitona lepidus*) resistance/tolerance – A progress report. *Proceedings of the New Zealand Grassland Association 63*: 177-181.

Eerens, J.P.J.; Hardwick, S.; Gerard, P.J.; Willoughby, B.E. 2005. Clover root weevil (*Sitona lepidus*) in New Zealand: the story so far. *Proceedings of the New Zealand Grassland Association 67*: 19-22.

Gerard, P.J.; Addison, P.J.; Hardwick, S.; Willoughby, B.E. 1999. Establishment of the invader: Insights into life history and biology of *Sitona lepidus* in the Waikato region of New Zealand. *Proceedings of the 7th Australasian Conference on Grassland Invertebrate Ecology*: 43-51.

Gerard, P.J. 2001. Dependence of *Sitona lepidus* (Coleoptera: Curculionidae) larvae on abundance of white clover *Rhizobium nodules*. *Bulletin of Entomological Research 91*:149.

Gerard, P.J.; Crush, J.R. 2003. Feeding habituation to red clover by clover root weevil adults. *New Zealand Plant Protection 56*: 129-132.

Gerard, P.J.; Crush, J.R.; Hackell, D.L. 2005. Interaction between *Sitona lepidus* and red clover lines selected for formononetin content. *Annals of Applied Biology 147*: 173-181.

Gerard, P.J.; Hackell, D.L.; Willoughby, B.E. 2004. Insights into clover root weevil larval feeding. pp. 49-57. *In*: Proceedings of the 8th Australasian Conference on Grassland Invertebrate Ecology. AgResearch Ltd, Lincoln, New Zealand.

Gerard, P.J.; Hackell, D.L. 2005. Leaf herbivory by clover root weevil adults – how much do they eat? *New Zealand Plant Protection 58*: 126-130.

Hardwick, S. 1998. Laboratory investigations into feeding preferences of adult *Sitona lepidus* Gyllenhal. *New Zealand Plant Protection 51*: 5-8.

Hardwick, S.; Harens, B. 2000. The behaviour of adult *Sitona lepidus* Gyllenhål (Coleoptera: Curculionidae) in response to white clover. *New Zealand Plant Protection 53*: 415-419.

Johnson, S.N.; Gregory, P.J.; Greenham, J.R.; Zhang, X.; Murray, P.J. 2006. Attractive properties of an isoflavonoid found in white clover root nodules on the clover root weevil. *Journal of Chemical Ecology 31*: 2223-2229.

Murray, P.J. 1996a. Influence of food source on feeding, longevity and fecundity of *Sitona flavescens* (Coleoptera: Curculionidae), a major pest of white clover in the UK. pp. 118-121. *In*: Recent research and development on white clover in Europe. Ed. Frame, J. REU Technical Series FAO, Rome.

Murray, P.J. 1996b. Evaluation of a range of varieties of white clover for resistance to feeding by weevils of the genus *Sitona*. *Plant Varieties Seeds 9*: 9-14.

Murray, P.J.; Clements, R.O. 1993. Feeding preferences among eleven cultivars of white clover (*Trifolium repens*) by adults of two species of *Sitona* weevil (Coleoptera: Curculionidae). *Tests Agrochemicals and Cultivars 14 (Annals of Applied Biology 122, Supplement)*: 134-135.

Murray, P.J.; Clements, R.O. 1994. Investigations of the host feeding preferences of *Sitona* weevils found commonly on white clover (*Trifolium repens*) in the UK. *Entomologia Experimentalis et Applicata 71*: 73-79.