

## Factors affecting clover root weevil (*Sitona lepidus*) numbers and clover production

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**Abstract** The impact of a range of simulated pasture management options on damage to white clover resulting from the presence of the clover root weevil was evaluated in a glasshouse experiment. Pasture management options included were irrigation, cutting regime, cultivar, soil fertility (Olsen P) level, and nitrogen application. Two different soil types were used. White clover growth was significantly enhanced by moist soil conditions, as were clover root weevil (CRW) larval numbers recovered per plot. Few (<1%) CRW larvae developed from the eggs (applied at 4000/m<sup>2</sup>) under dry soil conditions. Dry soil conditions dominated any effect that could be attributed to other treatments. More (c. 10.5%) CRW larvae developed under moist soil conditions, but clover plants tolerated this without significant losses to shoot production, although it did significantly ( $P = 0.01$ ) affect shoot/root ratio (on average 50% lower when CRW larvae were present). More clover dry matter was produced on mineral soil ( $P < 0.05$ ), when nitrogen was applied ( $P < 0.05$ ) and when the cultivar 'Challenge' was used instead of 'Prop' ( $P < 0.001$ ). Cutting regime had a minor influence ( $P < 0.10$ ) on clover production but the influence of Olsen-P level was not significant.

**Keywords** clover production; clover root weevil; irrigation; *Sitona lepidus*

### INTRODUCTION

The presence of clover root weevil (CRW, *Sitona lepidus* Gyllenhal) in New Zealand was first established in 1996 (Barratt et al. 1996). This initiated a wide-ranging research programme funded by government agencies and the private sector, as the potential economic damage to an important pasture species was considered to be very high. A number of key components of the pest needed to be understood, such as the species' initial distribution and rate of spread, its potential impact on the pastoral industry, and control and containment options. An initial survey established that CRW was distributed over a large area (Barratt et al. 1996), with primary infection sites being identified in Auckland and Tauranga (Willoughby & Addison 1997a). The combination of its geographic distribution and coexistence of all life stages in pastures (eggs on or close to the surface amongst the litter, soil-borne larval instars and pupae, and adults capable of flight), effectively ruled out any environmentally acceptable and economically viable eradication or containment options.

International literature (Byers & Kendall 1982; Mowat & Clawson 1996; Murray 1996) provided limited information on potential damage levels of CRW and anticipated behaviour patterns, but the descriptions proved different to those observed in New Zealand (Willoughby & Addison 1997b). The New Zealand environment was found to suit the requirements of CRW extremely well, with no effective population control mechanisms, few competing species and an abundant supply of white clover (*Trifolium repens* L.), the host species of choice. As a result, population densities were higher and individual female CRW produced more eggs than reported overseas (Willoughby & Addison 1997a). An initial impact assessment showed that CRW had the potential to completely eliminate white clover from pastures, and showed its preference for white clover over any other legume species. An examination of individual CRW specimens failed to show any evidence of diseases or parasitism (Barratt

et al. 1997) with the exception of a *Beauveria* fungus that infected and killed adult CRW (Willoughby et al. 1998). Natural *Beauveria* sp. populations present in New Zealand soils are apparently not able to prevent outbreaks of CRW. A promising parasitoid present in endemic CRW populations in Europe is being tested in quarantine in New Zealand (Goldson et al. 2001). Similarly, the search for CRW resistant/tolerant clover or productive non-host alternative legumes has yielded promising results (Eerens et al. 2001).

An examination of farms in affected areas has shown that while whole pastures, even whole farms, can be devoid of clover, apparently healthy clover can be found on the same farm along drains, raceways, lawns, etc., indicating that pasture management practices influence damage levels (J. P. J. Eerens unpubl. data). Even in the worst affected pastures some clover was found in dung patches. Dung patches are generally avoided by stock for several months and thus are not subjected to grazing pressure (J. P. J. Eerens unpubl. data).

A glasshouse experiment was conducted to simulate a range of environmental conditions based on field observations. In addition, management options for white clover were studied under varied CRW population densities. The results of this work are discussed in this paper.

## MATERIAL AND METHODS

### Experimental design

A glasshouse experiment was carried out at the Ruakura Research Centre during the spring and summer of 1997/98. A total of 128 crates, measuring 260 mm (deep) × 330 × 330 mm, were subdivided into two equal sized rectangular compartments (163 × 330 mm) by placing a hard plastic divider

down the middle of the crate. Holes in the bottom of the crates ensured drainage of excess water. These crates were partially filled with 160 mm of subsoil, on top of which 100 mm of topsoil was placed. Both were compacted to field density. Each compartment was then divided into two plots (no physical division). Clover was established from either stolon tips or seed in the centre of each plot.

### Treatments

The trial was of a complete factorial design with 2 soils × 2 cultivars × 2 cutting regimes × 2 nitrogen fertiliser levels × 2 phosphorus fertiliser levels × 2 CRW levels × 2 moisture levels × 4 replicates. The treatments applied in this experiment are listed in Table 1. The clover plants were first trimmed back 8 weeks after sowing and also at 11 and 14 weeks after sowing. At the time of the last cut, the spread of the plants was visually scored. This cover score was used as a covariance during the analysis. The soil fertility status of both the soils was determined at the time the crates were filled and the required amount of phosphorus fertiliser to raise the phosphorus level to achieve an Olsen P of 15 or 30, respectively, calculated.

Eggs were added 14 weeks after sowing, and for the following week all treatments were kept moist, without flooding the surface, to optimise hatching. Eggs were collected using two-tiered plastic containers, the tiers being separated by a fine mesh which allowed passage of eggs but not CRW adults. The adults fed and lived in the top tier and eggs dropped through the mesh to the bottom tier. Fertilised eggs change colour from white to black, and viability remains > 95% if they do not desiccate while stored for a short time (P. J. Gerard pers. comm.). Egg batches from a large number of CRW females were thoroughly mixed to ensure that each plot received an egg allocation of comparable make-

**Table 1** Treatments and the two levels they were applied at in the experiment as well as the point in time at which the treatments were initiated. CRW, clover root weevil.

Treatment	Level 1	Level 2	Start time
Soil type	Peat	Horotiu sandy loam	Sowing
Cultivar	'Challenge'	'Prop'	Sowing
Cutting regime	Hard (15 mm stubble)	Lax (35 mm stubble)	8 weeks after sowing
Phosphorus	Olsen P = 15	Olsen P = 30	11 weeks after sowing
Nitrogen	0 kg N/ha	200 kg N/ha	11 weeks after sowing
CRW	0 eggs	4000 eggs/m <sup>2</sup>	14 weeks after sowing
Soil moisture	Dry	Moist	15 weeks after sowing

up. Subsequently, the moisture (dry—lightly watered at 3-week intervals, or moist—watered daily) treatments were initiated. Every treatment combination was replicated four times.

The allocation of treatments over the plots and crates was not completely random. A single crate contained only one moisture treatment, while a single compartment (half crate) contained only a single P, N, soil or weevil treatment. The impacts of these adjustments to the randomisation were tested and found not to be significant. The trial was run over the summer months. Temperatures were between

20–25°C during the day and between 15–20°C overnight; very occasionally solar heating exceeded the capacity of the cooling system and daytime temperatures reached 30°C.

### Measurements

Plot dry matter cuts were taken 4 and 8 weeks after eggs were added; herbage was dried (overnight at 80°C) and weighed. After the final cut, the soil was washed over a sieve to collect CRW larvae and pupae, and all clover plant material (crown, shoots, stolons, and roots) was collected. Clover roots were

**Table 2** Main treatment effects on the number of larvae/m<sup>2</sup>, total clover shoot yield and clover shoot:root ratio.

Treatment	Larvae no./m <sup>2</sup>	Total clover shoot yield (g)	Shoot:root ratio
<b>Soil type</b>			
Peat	253	1.67	3.04
Horotiu	198	1.83	2.25
LSD <sub>0.05</sub>	45.9	0.14	0.53
Significance of difference	*	*	**
<b>Cultivar</b>			
‘Challenge’	225	1.94	2.49
‘Prop’	218	1.57	2.80
LSD <sub>0.05</sub>	45.7	0.13	0.53
Significance of difference	NS	***	NS
<b>Cutting regime</b>			
Hard	242	1.68	2.03
Lax	201	1.83	3.26
LSD <sub>0.05</sub>	53.3	0.16	0.90
Significance of difference	NS	NS	*
<b>Phosphorus (Olsen P)</b>			
15	238	1.74	2.87
30	205	1.77	2.42
LSD <sub>0.05</sub>	45.7	0.14	0.53
Significance of difference	NS	NS	NS
<b>Nitrogen (kg N/ha)</b>			
0	233	1.68	2.02
200	211	1.83	3.27
LSD <sub>0.05</sub>	45.7	0.14	0.54
Significance of difference	NS	*	***
<b>Clover root weevil (eggs/m<sup>2</sup>)</b>			
0	0	1.93	3.07
4000	444	1.58	2.22
LSD <sub>0.05</sub>	45.7	0.13	0.60
Significance of difference	***	***	***
<b>Soil moisture</b>			
Dry	36	1.69	2.33
Moist	410	1.82	2.96
LSD <sub>0.05</sub>	45.9	0.13	0.54
Significance of difference	***	NS	**

\*, \*\*, \*\*\* indicate  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively.

immediately washed and dried, and whole plants were stored in drum ethanol until they could be processed.

Clover roots (live and dead) were separated from shoots, dried (24 h at 80°C) and weighed. Data were analysed as a complete randomised block, using the cover score taken 14 weeks after sowing as a covariate. The shoot data were square-root-transformed. Back-transformed data are presented in Table 2.

## RESULTS AND DISCUSSION

The duration of the experiment was determined by the expected rate of larval development. The final harvest was timed well before adults were expected to emerge. Indeed, at the conclusion of the experiment there was no indication of adult CRW feeding in any of the treatments, neither was a single pupa found. Larval stages found varied from third instar to pre-pupa. The sieve used would have recovered second instar larvae but not first instars. In the absence of any second instar larvae, it was assumed that there were also no first instar larvae. These observations indicate that by the end of the experiment it was unlikely that any larvae had completed the development into an adult and had emerged and thus missed being counted. No larvae were found in any of the treatments that did not have CRW eggs applied to them, and neither were larvae found in 53 of the 256 plots to which eggs were added. Of these 53 plots, 51 were in the dry soil treatment. A total of 3150 larvae (11% of the eggs applied) were retrieved from 203 plots. This is only slightly lower than the *Sitona* numbers retrieved by Goldson et al. (1988).

Soil moisture had a significant ( $P < 0.001$ ) effect on larval numbers with around 12 times more larvae developing in moist soil (410/m<sup>2</sup>) than in dry soil (36/m<sup>2</sup>) (Table 2). Similar results were reported by Eerens et al. (1998) and Willoughby & Hardwick (1999). The results for soil type also confirmed the impact of soil moisture, with almost 30% ( $P < 0.05$ ) more larvae developing on roots in peat (253/m<sup>2</sup>) than in the mineral Horotiu soil (198/m<sup>2</sup>) (Table 2). This difference in larval numbers reflects the generally higher soil moisture retention capability of peat soils than mineral soils. Moisture in the soil, apart from providing the larvae with a medium for movement, prevents egg and larval desiccation by creating an environment of high humidity and buffering temperature fluctuations in the soil surface

layer (Addison et al. 1998). Larval development, especially development of the first instar larvae emerging from eggs, is critically dependent on adequate soil moisture to assist the movement from egg to a suitable food source. Goldson & French (1983) identified this phase as the most vulnerable in the life cycle of a related species, *Sitona discooides* Gyllenhal. Once a larva has started feeding on a clover nodule or root, soil moisture is less critical as the need for movement is reduced. Severe drought also affects clover growth, reduces its quality as a food source and causes a slowing down in CRW development.

Elevated soil moisture levels directly and indirectly affected CRW population dynamics. CRW larvae emerging from eggs were less likely to be desiccated and were more likely to successfully reach clover roots in moist soils compared with dry soils. Clover shoot yields were higher on moist plots ( $P < 0.001$ ) (Table 2) as the moist conditions provided a more suitable environment for white clover growth. A positive correlation between the population dynamics of free-living soil-dwelling plant parasites and the abundance and quality of a food source has been reported for nematodes (Yeates 1984) and *Sitona* (Muller 1963). There are similarities in the driving forces behind the population dynamics of the early larval stages of CRW and nematodes (B. Willoughby pers. comm.). Soil moisture had such a dominant effect on both clover growth and larval numbers that it was impossible to determine whether clover root quality or soil moisture was the factor determining larval numbers. Larval numbers were determined predominantly by soil moisture (or forage quality) and to a lesser extent by clover shoot production, root weight, and plant cover at the start of the experiment. None of the other treatments had a significant effect on larval numbers.

Clover productivity was higher for the cultivar 'Challenge' ( $P < 0.001$ ), when nitrogen was applied ( $P < 0.05$ ), when plants were cut in a more lax manner and on mineral rather than peat soil ( $P < 0.1$ ). 'Challenge' is an erect growing medium to large leaf variety more suited to the rotational grazing management simulated in this experiment, while 'Prop' is a prostrate and small leaf variety more suited to a set stocking regime. The yield difference observed between the cultivars is likely the result of the cutting regime applied. While white clover has the ability to fix nitrogen, in the presence of a readily available pool of mineral nitrogen, clover will defer from fixing nitrogen.

Clover shoot:root ratio was on average highest on moist soils, when nitrogen fertiliser was added and in the absence of CRW larvae. The impact on the shoot:root ratio of either lack of nitrogen fertiliser or addition of CRW larvae were similar in magnitude. This could reflect the preference of early CRW larval stages for the nitrogen-fixing nodules of white clover (Gerard 2001), which causes effects similar to nitrogen deficiency. No assessments of nodule damage were attempted as it was assumed that significant nodule damage would be reflected in reduced ability to produce shoot material.

## CONCLUSION

Clover root weevil larval development appears to be positively influenced by the availability of white clover and moist soils (created either through regular watering or higher moisture retention). This could be due to the fact that moist soils provide a benign environment for larval and clover development. Higher CRW loading on clover plants growing in moist soils did not cause a significant loss in productivity. None of the other factors tested had a significant influence on larval numbers.

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