Effects of clover root weevil and moisture stress on subterranean, balansa, Persian, arrowleaf and white clovers

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Abstract

The effect on plant growth of clover root weevil (CRW) larval feeding on nodules and roots was examined for the annual clovers, subterranean cv. Leura, balansa cv. Bolta, arrowleaf cv. Arrowtas and Persian cv. Mihi, and white clover cv. Tribute. Mini-swards of each clover species were used in a glasshouse experiment, with half of these exposed to CRW larval feeding. A moisture stress treatment was imposed as a sub-treatment on half the swards with and without CRW. CRW larval feeding had no effect on shoot growth, but reduced root weights significantly with most effect on white clover. More CRW larvae were recovered from core samples taken from white clover than from the annual clovers. Effects of CRW were generally more severe in the well-watered swards. It is concluded that the relative tolerance to CRW shown by the annuals combined with the negative effects of summer drought on CRW populations should mean that CRW does not pose a major threat to dryland pastures based on annual legumes.

Keywords: *Sitona lepidus*, root herbivory, annual clovers, drought

Introduction

The spread of clover root weevil (*Sitona lepidus*, CRW) through moist, white clover-based pastures in New Zealand has had a detrimental effect on the clover content, and associated nitrogen (N) fixation of these swards (Eerens *et al*. 2005). Comparatively little research has been done on the impact of CRW on annual legumes which are important where regular moisture deficits limit persistence of white clover. Crush *et al*. (2007) reported the effect of CRW larval feeding on roots and nodules of subterranean clover (*Trifolium subterraneum*), suckling clover (*T. dubium*), clustered clover (*T. glomeratum*) and striated clover (*T. striatum*), that are naturalised on shallow, stony lowland soils, dry north-facing hills and the inland South Island arid soils (Boswell *et al*. 2003). Growth of suckling, striated and clustered clover was significantly reduced by CRW, approaching levels of damage recorded in white clover. Nitrogen fixation in suckling and clustered clovers was also reduced. Growth and nitrogen fixation of two subterranean clovers (cv. Denmark, cv. Leura) were not changed significantly by CRW larval feeding. It was suggested that the relatively high level of the isoflavonoid biochaninA that was present in subterranean clover roots might explain the species tolerance of CRW. The objective of this work was to study the effect of CRW on named cultivars of four annual clovers which might be sown in improved dryland pastures.

Materials and Methods

Te Rapa silt loam soil was collected from run out pasture near Hamilton and sieved through a 10 mm mesh to break up lumps. The soil was packed to 17 cm depth at 0.85 volume weight (the subsoil density for this soil) in 33 cm square plastic boxes with drainage holes in their bases. This was covered with an 8 cm layer of soil that had been premixed with calcium phosphate and potassium sulphate to meet fertiliser recommendations for sheep and beef farms (Overseer^{R} 2008) and packed to the topsoil density of 0.8.

Seed of cv. Leura subterranean clover (*Trifolium subterraneum*), cv. Bolta balansa clover (*T. michelianum*), cv. Mihi Persian clover (*T. resupinatum*), cv. Arrotas arrowleaf clover (*T. vesiculosum*) and cv. Tribute white clover (*T. repens*) was germinated in seedling trays and inoculated with *Rhizobium*. Twenty five days after germination, miniswards of each clover were established by transplanting nine healthy seedlings of each clover into individual boxes with 20 replicates of each clover type. The treatments were arranged in a row/column design in a temperature-controlled glasshouse with mean day and night temperature of 21.4 and 14.9ºC respectively. Soil moisture was maintained at adequate levels by daily watering, and swards were weeded regularly. Seven weeks after transplanting when the plants were well-nodulated and growing vigorously, half the replicate boxes were inoculated with 90 CRW eggs and 10 newly-hatched CRW larvae per box and the remaining10 replicate boxes per cultivar were maintained as the CRW-free controls. Two weeks later all the swards were cut to 2.5 cm height, and the clippings oven-dried and weighed. Soil water contents were measured gravimetrically on soil cores on six extra boxes set up for this purpose and growing subterranean clover. After 3 weeks regrowth, the water input to half the CRW boxes and half of the controls was reduced to 1 litre weekly for 3 weeks and the rest continued to receive 1 litre per box daily. Over this period soil moistures in the stressed treatments declined to 17%, and then the soil was fully rewetted to prevent plant mortality, and the dry-down treatment repeated. Soil water contents in the watered boxes ranged between 30.0 and 31.5%.

The experiment finished 14 weeks after establishment. The shoots were cut off at ground level, oven-dried and weighed. Two cores (65 mm diam. x 140 mm depth), each over a single plant, were taken per box, and CRW larvae and plant roots recovered. Roots were washed clean, oven dried and weighed. Data were analysed using the General Linear Model for ANOVA (MINITAB® 2004, version 14-2. Minitab Inc., USA). Least significant differences (LSD) were calculated at the 0.05 probability level.

Results

At the first harvest, 2 weeks after inoculating the plants with CRW, there was no significant difference in shoot yield between CRW-inoculated and CRW-free plants. However, there were highly significant (P<0.001) differences in shoot yield among the clovers. Mean values (kg DM/ha) were: sub clover 3305; balansa 9015; Persian 6445; arrowleaf 3520; white 3680; $LSD_{0.05}$ between species = 650.

At the second harvest, after 6 weeks growth with CRW and 4 weeks of two moisture levels, the reduction in shoot DM in the plus CRW treatments relative to no CRW was not significant (P=0.07), but the effects of clover type and soil moisture supply on shoot DM were both significant (P<0.001) (Table 1). Arrowleaf clover had the highest shoot DM yield and balansa had the lowest. Moisture stress without CRW reduced shoot DM of sub clover by 17% and reduced shoot DM of the other clovers by 21-26%. The moisture treatment × clover type interaction was significant (P<0.001), but the moisture × CRW, and clover × CRW interactions were not significant.

Root DM differed among the clovers (P<0.001), and was greatest in arrowleaf clover. Root DM was significantly (P<0.004) lower in the plus CRW than in the no CRW treatment, and in the moisture stress compared with the well-watered treatment (P<0.031) (Table 2). In the well-watered treatment, CRW reduced root DM of sub clover by 5%, of balansa, Persian and arrowleaf clover by 20-27%, and root DM of white clover by 63%. In the moisture stress treatment, CRW reduced root DM of white clover by 39%, but had little effect on root DM of the other clovers. The moisture treatment x CRW interaction on root DM was significant (P<0.01), but the moisture x clover and CRW x clover interactions were not significant.

The number of larvae recovered from the soil after the final harvest was significantly (P<0.01) higher in white clover than for the annual clovers as a group, and there was no difference between watered and moisture stress treatments. Values were: white clover 2.25 larvae/core; sub clover 0.23/core; Persian clover 0.78/core; balansa 1.26/core; and arrowleaf 1.88/core. Almost all the larvae present were final instars. However, CRW development was more advanced on white clover (P<0.012) with 55% of CRW recovered being pupae or adults compared with 18% from the annual clovers.

Table 1 Shoot dry matter (kg/ha) for five clovers with adequate moisture or water-stressed and with or without clover root weevil feeding on nodules and roots. $LSD_{0.05}$ for water = 378, clover species = 596.

Clover	Watered		Stressed	
	+CRW	-CRW	+CRW	-CRW
Sub clover	4624	4751	4343	4397
Balansa	970	1581	874	504
Persian	4360	5715	1815	2272
Arrowleaf	5104	5697	3462	3521
White clover	3888	4518	2731	2514

Table 2 Root dry matter (kg/ha) for five clovers with adequate moisture or water-stressed and with or without clover root weevil feeding on nodules and roots. $LSD_{0.05}$ for water = 271, CRW = 273, clover species = 427.

Clover	Watered		Stressed	
	+CRW	-CRW	+CRW	-CRW
Sub clover	2345	2465	1984	2053
Balansa	1416	1938	1714	1460
Persian	2342	2960	2368	2222
Arrowleaf	3530	4502	3659	3536
White clover	867	2327	1058	1725

Discussion

In this experiment CRW larval feeding reduced root weights significantly but the root damage was not sufficiently severe to have a statistically significant effect on shoot weights. White clover lost much more root mass due to CRW larval feeding than did subterranean clover, with the other annuals showing intermediate levels of damage. The highest number of CRW larvae recovered was from white clover, confirming its status as the preferred host of CRW, and relatively few larvae were recovered from sub clover. The results confirm earlier evidence for the relative tolerance of sub clover to CRW (Hardwick 1998; Crush *et al*. 2007). Roots of Leura sub clover contain high levels of the isoflavone biochaninA that has been implicated in the CRW tolerance of this cultivar (Crush *et* *al*. 2007) and of red clover (Crush *et al*. 2006).

The effects of CRW larvae on root and shoot DM were more pronounced in the well-watered treatments. This suggests that CRW larvae are better adapted to the moist conditions where its preferred host, white clover, thrives. The observed low proportion of reproductive adults and increased mortality amongst eggs and newly-hatched larvae in hot dry summers (Addison *et al* 1998; Gerard & Arnold 2002), suggest that CRW is unlikely to be an aggressive pest under drier conditions.

Because of edge effects, e.g. reduced competition for light, the yields from the mini-swards may be inflated compared with plants grown more extensively. Taking this into account there were still some striking differences between cultivars in shoot DM yields. Balansa and Persian clovers had the highest and identical yields, but balansa produced 90% of its total yield by the first cut compared with 65% in Persian clover. In field trials, balansa has demonstrated rapid establishment and high growth rates (Hyslop *et al*. 2003). Better regrowth of balansa may have been achieved with an earlier first cut. Sub clover, arrowleaf and white clover had similar proportionate yields in both cuts.

The CRW larvae had no dietary choice in the experiment, but given the very small size of newly hatched CRW larvae, and their presumably restricted mobility in soil, and that the species feeds solely on legumes, they may have little choice of diet in the field. The results show that well-established annual clovers, especially sub clover are more tolerant of CRW larval damage than is white clover. The degree of adult feeding and oviposition on annual legumes may be determined by the frequency of white clover in the sward. Therefore, the relationships between the legume composition of a sward and risk posed by CRW adults and larvae on establishing annual clovers still need to be determined. The content of perennial clovers, especially white clover will be critical in determining the CRW population that survives over dry summer conditions to attack annual clovers establishing in autumn.

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REFERENCES

Addison, P.J.; Willoughby, B.E.; Hardwick, S.; Gerard, P.J. 1998. Clover root weevil: observations on differences between 1997 and 1998 summer populations in the Waikato. *Proceedings of the 51st New Zealand Plant Protection Conference*: 1-4.

Boswell, C.C.; Lucas, R.J.; Lonati, M.; Fletcher, A.; Moot, D.J. 2003. The ecology of four annual clovers adventive in New Zealand grasslands. *Legumes for Dryland Pastures. Grassland Research and Practice Series 11*: 175-184.

Crush, J.R.; Gerard, P.J.; Rasmussen, S. 2006. Formononetin in clovers as a feeding deterrent against clover root weevil. *Advances in Pasture Plant Breeding. Grassland Research and Practice Series 12*: 135-139.

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

Gerard, P.J.; Arnold E.D. 2002. Influence of climate regime on clover root weevil adult survival and physiology. *New Zealand Plant Protection 55*: 241-245.

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.

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

Hyslop, M.G.; Slay, M.W.A.; Moffat, C.A. 2003. Dry matter accumulation and sheep grazing preference of six winter active annual legumes. *Legumes for Dryland Pastures. Grassland Research and Practice Series 11*: 117-122.

Overseer[R] 2008. Overseer nutrient budgets. www.agresearch.co.nz/overseerweb