

## YIELD RESPONSE OF LEGUME SEEDLINGS TO SIMULATED INSECT COTYLEDON DAMAGE AND NUTRIENT AVAILABILITY

C.M. FERGUSON, J.A. SHAND and A.A. EVANS

*AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel*

### ABSTRACT

Seedlings of nine legume species grown in pots were subjected to simulated insect defoliation by removing 0, 0.5, 1, 1.5 or 2 cotyledons. Foliage and root yields were measured after 8 weeks. The reaction of white clover, Caucasian clover (*T. ambiguum*), and lucerne (*Medicago sativa*), to the same levels of defoliation was further investigated when grown under three balanced nutrient levels but without nitrogen. Foliage and root production and the amount of nitrogen fixed were measured 8 weeks after defoliation. Growth of all legume species decreased as simulated damage increased but some evidence suggested that the response varied between species. No association between simulated damage and nitrogen fixation was demonstrated but nutrient availability was shown to be of greater importance to plant growth than damage to cotyledons.

**Keywords:** legume seedlings, simulated insect damage

### INTRODUCTION

The detrimental effect of cotyledon excision, either natural or artificially imposed, on subsequent plant growth and production has been documented for several plant species. Bignoli (1950) found that early defoliation of lucerne (*Medicago sativa* L.) resulted in reduced plant size and yield which persisted for 5 months, Killeen and Larson (1968) found cotyledon excision to reduce the rate of axis growth in *Pisum sativum* and Wassermann and Kruger (1983) showed a relationship between increasing severity of damage to cotyledons of *Lupinus albus* and reduction in plant height, leaf number/plant and yield. Simulated insect damage to cotyledons of white clover (*Trifolium repens* L.) has been shown in laboratory and field studies to be associated with reduced plant survival, yield loss and a reduction in the size of root nodules (Barratt 1980, 1985) and damage by broad-nosed weevils (Curculionidae: Brachycerinae: Entimini) in Otago tussock grassland has been shown to translate into major yield losses persisting for several years after establishment (Barratt *et al.* 1992). The same native weevils have been shown to feed on a range of pasture legumes (Barratt *et al.* 1993) and to reduce establishment and production of Caucasian clover (*Trifolium ambiguum* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) (Ferguson and Evans 1994). Barratt and Jones (1994) found that the partial or complete defoliation of white clover cotyledons by broad-nosed weevils did not reduce subsequent plant growth to a greater or lesser extent than simulated defoliation and concluded that weevil feeding damage simulated by clipping provides a representative measure of plant response to insect damage.

This study investigated the effect on plant production of simulated insect damage to cotyledons of a range of pasture legumes and on the production and nitrogen fixation of white clover, Caucasian clover and lucerne when grown in a glasshouse at three fertility levels. The aim was to determine if the legume species tested were all affected to a similar extent by simulated insect feeding or whether their reactions differed, information that would assist in selecting suitable legumes for oversowing in insect-damage prone areas.

*Proc. 48th N.Z. Plant Protection Conf. 1995: 213-218*

## METHODS

### Experiment 1

Soil, collected from Mt McKay station, near Middlemarch, was steam sterilised and used to fill 450 "PB 1.5" containers. For each legume species tested (Table 2) ten seeds were sown into each of 50 bags on 26 January 1994. The bags were randomly positioned within the trial area, located in an outside nursery, and were moved to new random positions after 3 and 5 weeks. The legume seedlings were inoculated with the appropriate strain of rhizobia 2 weeks after sowing. The day following inoculation the seedlings were thinned to five per bag and defoliation treatments were imposed by removal of 0, 0.5, 1, 1.5 or 2 cotyledons with scissors. Intact seedlings were used as an untreated control treatment. There were ten replicate bags for each treatment. Three weeks after sowing an aqueous solution of sodium molybdate, ibex and gypsum was applied at a rate equivalent to 350 kg molybdate sulphur superphosphate/ha to the soil surface. The plants were exposed to normal rainfall and otherwise watered as required. After 8 weeks the plants were harvested by removing them from the bags and extracting the roots from the soil by hand sorting and washing with water. The foliage and roots were separated and dried in an oven at 80 °C for 12 h before weighing. The dry weights were log transformed and analysed by analysis of variance. The log transformed data were also analysed by an additive main effects multiplicative interaction model (A.M.M.I.) model (Kempton 1984) to detect differences between species.

### Experiment 2

Lucerne, white clover and Caucasian clover seeds were sown in root trainers containing inert growing media, (2:1 vermiculite:perlite for lucerne and horticultural sand for other species). Two seeds per cell were sown but were thinned to one seedling per cell when the defoliation treatments, as for Experiment 1, were applied 7 days after emergence. Intact seedlings were used as controls. Sixteen seedlings, each in their own cell, made up one plot and each defoliation treatment was replicated 15 times. The seedlings within five replicates of each defoliation treatment were grown under one of three nutrient regimes arbitrarily termed high (adequate nutrients for plant growth), medium or low. In each case the nutrients were applied in a balanced solution containing all necessary major and minor elements with the exception of nitrogen (Table 1). The solutions were applied at a rate of 10 mls twice a week for 8 weeks. The plants were watered daily with reverse osmosis purified water. After 8 weeks the plants were harvested by washing the growing media away from the roots. The foliage and roots were oven dried at 80 °C for 12 h and weighed separately before being recombined, ground and submitted to the AgResearch Soil Fertility Service for estimation of nitrogen composition by Kjeldahl digestion's and colorimetric analyses. The dry weights and nitrogen content of the plants were analysed by analyses of variance.

**TABLE 1: Amount of nutrient (mg) per litre of nutrient solution.**

	K	P	S	Mg	Ca	Na	Cl	Fe	B	Mo
high	120.998	41.34	27.222	9.724	46.776	2.296	87.226	0.344	0.3	1.5E-03
medium	24.20	8.27	5.44	1.94	9.36	0.46	17.45	0.068	0.06	0.3E-03
low	1.21	0.4134	0.2722	0.00972	0.4677	0.02296	0.87226	0.00344	0.003	1.5E-05

## RESULTS

### Experiment 1

All species except lucerne showed progressive decreases in foliage production from the untreated plants to 1 and both cotyledons removed although the decrease between 0 and 1 cotyledon was significant only for white clover and crown vetch ( $P < 0.05$ ) (Table 2). The difference between untreated plants and those with both cotyledons removed was significant for all species ( $P < 0.05$ ) except alsike clover

( $P < 0.1$ ) and lucerne. The removal of 0.5 cotyledons only had a marked adverse effect on white clover foliage production ( $P < 0.1$ ) and 1.5 cotyledons removed did not in any case significantly decrease production to greater extent than 1 cotyledon removed but foliage production by birdsfoot trefoil and crown vetch was significantly lower than for untreated plants. Root production (Table 2) followed a similar pattern to foliage production except the root production by white clover was significantly reduced by the removal of 0.5 cotyledons or more ( $P < 0.05$ ) and Caucasian clover, Maku lotus, crown vetch and perennial lupin by the removal of one or more cotyledons ( $P < 0.05$ ).

The A.M.M.I. analysis suggested that the difference in both foliage and root production averaged for alsike clover, red clover and lucerne subjected to no damage, 0.5 and 1 cotyledon removed (foliage = 0.93 g, roots = 0.32 g) and those plants subjected to 1.5 and 2 cotyledons removed (0.75 g and 0.25 g respectively) was less than the difference between the same groups of damage levels for crown vetch and perennial lupin (foliage 0.96 g and 0.39 g, roots 0.40 g and 0.16 g respectively) ( $P < 0.05$ ).

**TABLE 2: Foliage and root production (back transformed values) (g/pot) of nine legume species 8 weeks after sowing.**

		Cotyledons removed				
		0	0.5	1	1.5	2
white clover	foliage	1.13	0.68	0.63	0.66	0.41
( <i>Trifolium repens</i> )	roots	0.30	0.19	0.17	0.17	0.10
Caucasian clover	foliage	0.68	0.66	0.40	0.44	0.37
( <i>Trifolium ambiguum</i> )	roots	0.40	0.43	0.27	0.27	0.21
alsike clover	foliage	1.13	1.04	0.84	1.06	0.64
( <i>Trifolium hybridum</i> )	roots	0.41	0.36	0.30	0.36	0.22
red clover	foliage	1.13	0.88	0.84	1.06	0.43
( <i>Trifolium pratense</i> )	roots	0.34	0.24	0.24	0.31	0.13
lucerne	foliage	0.79	0.87	0.87	0.78	0.72
( <i>Medicago sativa</i> )	roots	0.34	0.34	0.36	0.28	0.27
birdsfoot trefoil	foliage	1.33	1.30	1.12	0.73	0.51
( <i>Lotus corniculatus</i> )	roots	0.33	0.35	0.28	0.16	0.13
Maku lotus	foliage	1.71	1.62	0.99	1.20	0.50
( <i>Lotus pedunculatus</i> )	roots	0.28	0.24	0.16	0.17	0.09
crown vetch	foliage	0.50	0.56	0.24	0.24	0.08
( <i>Coronilla varia</i> )	roots	0.23	0.24	0.14	0.19	0.05
perennial lupin	foliage	2.40	2.43	1.92	1.59	0.70
( <i>Lupinus alpinus</i> )	roots	0.95	0.93	0.59	0.46	0.25
<sup>1</sup> LSR ( $P < 0.05$ )	foliage	1.78				
	roots	1.56				

<sup>1</sup> least significant ratio

## Experiment 2

The patterns of foliage and root production associated with the simulated damage treatments were similar at all three fertility levels for each legume species although the magnitude of the low fertility data was much smaller than for the two higher levels (Fig. 1).

For all three legumes the amount of percent nitrogen content of plant weight was not affected by the simulated insect damage treatments within any of the fertility treatments. The percent nitrogen, averaged over the damage treatments (Table 3), increased significantly as the concentration of the nutrient solution increased irrespective of the amount of simulated damage (white clover  $P < 0.01$ , Caucasian clover and lucerne  $P < 0.05$ ) (Table 3).

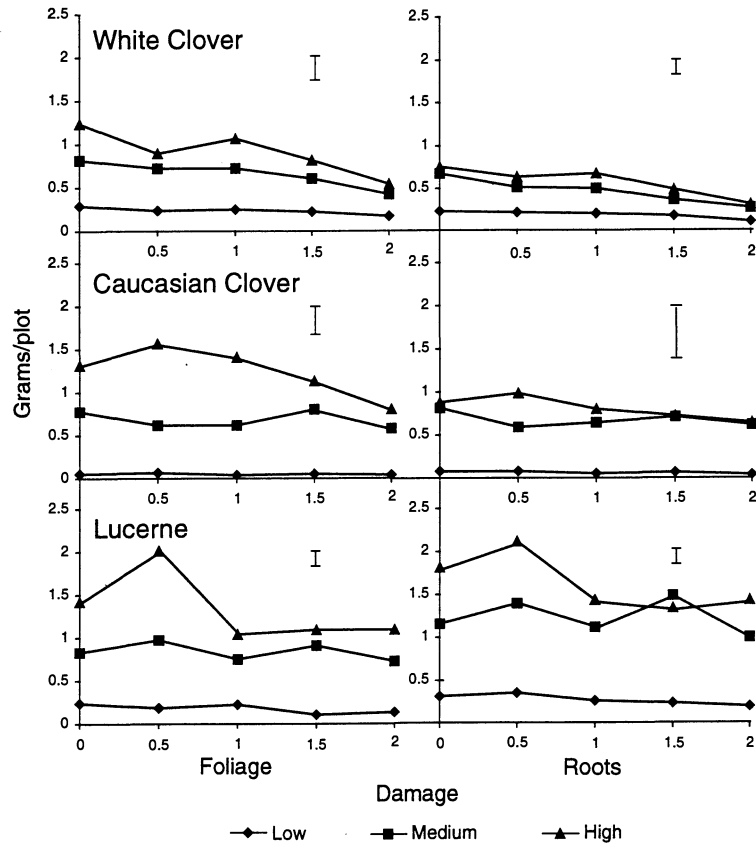


FIGURE1: Foliage and root production of white clover, Caucasian clover and lucerne (g/plot) 8 weeks after sowing. Error bars = LSD (P<0.05)

TABLE 3: Percentage nitrogen averaged over all damage treatments.

	Low	Medium	High	LSD (P<0.05)
white clover	1.717	3.180	3.809	0.264
Caucasian clover	1.889	2.400	2.766	0.269
lucerne	2.914	3.882	4.146	0.232

DISCUSSION

The A.M.M.I. model suggested that alsike clover, red clover and lucerne were less affected by cotyledon damage than perennial lupin and crown vetch. Taking into account the variation in production of species between Experiments 1 and 2 and the influence of environmental factors on legume production following seedling damage (Barratt 1985) this finding requires further investigation to establish its importance.

Both experiments indicated that although all species tested, with the exception of lucerne in Experiment 1, produced decreasing amounts of foliage and roots as simulated damage increased, most species tolerated the removal of half a cotyledon

and showed little if any decrease in either root or shoot production. This concurs with the findings of Barratt (1985), who showed the loss of half a cotyledon did not affect the subsequent production of white clover. The removal of an entire cotyledon in Experiment 1 did, however, reduce the production of foliage and roots of white clover and crown vetch, and root production of Maku lotus and perennial lupin which again concurs with the results of Barratt (1985) for white clover. In Experiment 2 the removal of one cotyledon from white clover did not significantly affect the subsequent production. Apparent disparities between the results from Experiments 1 and 2 are also evident for Caucasian clover and lucerne but this is not surprising as Barratt (1985) found that the effect of similar amounts of damage varied between years depending on climate. The plants, in this investigation, were grown under very different conditions in the two experiments. The absence of a response to damage by lucerne in Experiment 1 is very likely due to an overriding effect of a low soil pH (5.1) (W. Lowther pers. comm.).

The results of this investigation and Barratt's work demonstrate that other factors have major influences on the potential effect of damage to cotyledons. It was suspected that one of these may have been nutrient availability and that if plants were under nutrient stress then the effect of insect damage to cotyledons would be accentuated. This was not the case. In this investigation the greatest reductions occurred where nutrients were unlikely to be limiting and became progressively smaller as nutrients became limiting. In the low fertility treatment it is probable that the scarcity of nutrients limited the growth of plants and that this factor over rode any effect the simulated damage treatments may have, in a way similar to the overriding effects of climate as suggested by Barratt (1985). Nutrient availability is not generally limiting in field situations but deficiencies of one or more nutrients are common. If these deficiencies severely limit plant growth it is possible that insect damage will not further reduce it.

The reduction in root nodule number reported by Barratt (1985) and assumed reduction in nitrogen fixation associated with increasing cotyledon damage was not repeated in this investigation. Nodule size would also have been limited by nutrient scarcity and this in turn probably limited the amount of nitrogen fixed (Sprent and Minchin 1983). The high and medium fertility levels did not limit plant growth to the same extent, nor presumably nodule size although this was not measured.

#### CONCLUSION

The legume species tested were all adversely affected by simulated insect damage when one or more cotyledons were removed but the loss of half a cotyledon generally had little effect. There was some evidence that the response of the legume species tested to cotyledon damage differed but the importance of this was not established.

#### ACKNOWLEDGEMENTS

This investigation was sponsored by The Foundation for Research, Science and Technology. The authors thank Mr. P.D. Johnstone for the statistical analyses of the data.

#### REFERENCES

- Barratt, B.I.P., 1980. Effect of simulated insect damage to white clover seedlings. *Proc. 33rd Weed and Pest Control Conf.*: 49-51
- Barratt, B.I.P., 1985. Effect of cotyledon damage on nodulation and growth of white clover oversown into native grassland in Central Otago, New Zealand. *Proc. 4th Australasian Grassl. Invert. Ecol. Conf.*: 127-132
- Barratt, B.I.P., Ferguson, C.M., Jones, P.A. and Johnstone, P.D., 1992. Effect of native weevils (Coleoptera: Curculionidae) on white clover establishment and yield in tussock grassland. *N.Z. J. Agric. Res.* 35: 63-73
- Barratt, B.I.P., Jones, P.A., and Ferguson, C.M., 1993. Screening legumes for susceptibility to broad-nosed-weevils (Coleoptera: Curculionidae). *Proc. 6th Australasian Grassl. Invert. Ecol. Conf.* : 189-194

- Barratt, B.I.P. and Jones, P.A., 1994. A comparison between real and simulated insect defoliation of white clover seedlings, and the effects of timing and the degree of damage. *Proc. 47th N.Z. Plant Protection Conf.* : 201-205
- Bignoli, D.P., 1950. The effect of early defoliation on lucerne seedlings. *Br. Grassl. Soc. J.* 5: 281-286
- Ferguson, C.M. and Evans, A.A., 1994. Field screening of seedling dryland legume species to insect feeding. *Proc. N.Z. Sust. Land Manag. Conf.* 1994: Lincoln, Canterbury. 227-231
- Kempton, R.A., 1984. The use of biplots in interacting variety by environment interactions. *J. Agric. Sci. Camb.* 103: 123-135.
- Killeen, L.A. and Larson, L.A., 1968. The effect of cotyledon excision on the growth of pea seedlings. *Am. J. Bot.* 55: 961-965.
- Sprent, J.L. and Minchin, F.R., 1983. Environmental effects on the physiology of nodulation and nitrogen fixation. *In: Temperate Legumes. Physiology, Genetics and Nodulation.* Jones, D.G. and Davies, D.R. (Eds). Association of Applied Biologists. 442p.
- Wasserman, V.D. and Kruger, A.J., 1983. Some effects of simulated cotyledon damage during the seedling stage on growth and production of *Lupinus albus* L. *Cv. Ultra. Agroplanta* 15(2): 29-33