A COMPARISON BETWEEN REAL AND SIMULATED INSECT DEFOLIATION OF WHITE CLOVER SEEDLINGS, AND THE EFFECTS OF TIMING AND DEGREE OF DAMAGE

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SUMMARY

The effect on plant growth of simulated insect defoliation of white clover seedlings by clipping cotyledon leaves, was compared with weevil feeding damage in the glasshouse. A second experiment investigated the response of white clover seedlings to different times and levels of severity of defoliation. No differences in plant growth were found between the simulated and actual defoliation treatments irrespective of severity of defoliation, suggesting that simulated damage is a reliable representation of actual insect defoliation. Plant yields 10 and 15 weeks after treatment were progressively reduced with increasing degrees of defoliation. The interval between cotyledon emergence and defoliation produced no overall differences in yield 15 weeks after treatment, although earlier defoliations caused greater yield reductions after 8 weeks.

Keywords: white clover, *Trifolium repens*, cotyledon seedlings, weevil damage, simulated damage

INTRODUCTION

Trials in Otago tussock grassland have identified broad-nosed weevils (Curculionidae: Brachycerinae: Entimini) to be significant pests of introduced white clover (*Trifolium repens*) seedlings in the cotyledon stage (Barratt 1981). This early damage has been shown to translate into major yield losses persisting for several years after establishment (Barratt *et al.* 1992). Barratt and Johnstone (1984) found that establishment failure and dry matter yield reduction were higher than could be accounted for by seedling mortality alone, suggesting that sub-lethal cotyledon defoliation reduced subsequent survival and growth of seedlings. In a glasshouse experiment, Barratt (1980) found that cotyledon excision soon after germination reduced root and shoot dry weights of white clover plants by up to 55% three months after treatment. Artificial defoliation of white clover seedlings in the laboratory and in the field reduced the percentage nodulation, survival and dry matter production of plants (Barratt 1985).

Simulated insect defoliation is often used to assess the plant production losses caused by real insect defoliation in field conditions since it offers the advantages of convenience, ease of replication, and precision. Most experimental work using simulated defoliation to imitate the effects of chewing insects (eg. Dunning and Winder 1971; Bignoli 1950; Knight and Holloway 1962) has assumed that mechanical removal of plant tissue could be reproduced by clipping. This assumption, however, has rarely been substantiated.

Using real and simulated grasshopper grazing of wheat seedlings, Capinera and Roltsh (1980) questioned the reliability of using simulated damage techniques to predict the loss of production. They found that the regrowth of wheat seedlings exposed to insect defoliation was reduced compared with those subjected to the equivalent levels of manual clipping in both laboratory and greenhouse trials. They speculated that a factor associated with grasshopper feeding disrupted the normal

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wound response of wheat seedlings when defoliation was severe, resulting in impaired regrowth potential. Such a factor may be salivary in origin (Dyer and Bokhari 1976), and apparently the effect on the plant depended upon the level of defoliation. Sucking insects often introduce substances into their host plants bringing about physiological changes which cannot be simulated (Capinera and Rolsh 1980).

The objectives of the two experiments reported here were firstly to determine whether simulated broad-nosed weevil damage to white clover seedlings is a reliable method of assessing plant response to insect damage which occurs in the field, and secondly to investigate the relationship between timing and extent of white clover cotyledon leaf removal and subsequent plant growth.

METHODS

Experiment 1

The experiment was carried out in a glasshouse heated to 15-25°C. Single seeds of "Grasslands Huia" white clover were sown in 4 cm diameter x 8 cm long plastic open ended tubes containing soil to facilitate the removal and analysis of roots at a later stage. After germination, seedlings at the same stage of cotyledon expansion were selected and embedded in groups of six into plastic containers filled with soil, with each container assigned to one of seven treatments. The treatments were carried out as soon as the cotyledon leaves had fully opened as follows:

- (1) one cotyledon leaf removed by clipping,
- (2) one and a half cotyledon leaves removed by clipping,
- (3) both cotyledon leaves removed by clipping,
- (4) one cotyledon leaf removed by weevil feeding,
- (5) one and a half cotyledon leaves removed by weevil feeding,
- (6) both cotyledon leaves removed by weevil feeding,
- (7) control, no leaves removed.

The weevil defoliation treatments were carried out by placing the portions of the cotyledons to be removed through a slot into a gelatine capsule containing two adult broad-nosed weevils (*Nicaeana cinerea* Broun). Vials were left in position for 24 h and if the required amount of feeding had not occurred the seedling was discarded and replaced. If placing the vial in position caused any damage to the seedling, it was also replaced.

The treatments were replicated eight times, and the 56 containers were completely randomised and systematically shifted in a uniform pattern every 2 days, so each pot spent an equal amount of time in each position during the trial. After 10 weeks, three plants were removed from each container and harvested, and after 15 weeks the remaining three plants were harvested. The maximum root and shoot lengths were measured, then oven dried overnight and weighed.

The results were analysed by ANOVA using the means of the three plants from each pot at each harvest to examine differences between types of defoliation and level of defoliation.

Experiment 2

The trial was carried out in a glasshouse maintained at 18-25°C. Pots, 15 cm diameter, containing sterilised soil were sown with "Grasslands Huia" white clover seeds. Rhizobial inoculant was watered into the soil after germination. Six seedlings at the same stage of development with cotyledon leaves fully emerged were retained in each pot and the rest removed.

Each pot was then assigned to one of the following treatments as follows:

- (1) one cotyledon leaf removed by clipping,
- (2) one and a half cotyledon leaves removed by clipping,
- (3) both cotyledon leaves removed by clipping,
- (4) control, no leaves removed.

The clipping treatments were carried out on 1, 2, 4, 8 or 16 days after the cotyledon leaves had fully opened. Each treatment combination was replicated ten times and the pots were located in a randomised block design in the glasshouse.

Eight weeks after the cotyledons had opened three alternate plants from each pot were harvested at soil level, dried and weighed. The remaining three plants were harvested, dried and weighed after 15 weeks.

The results were analysed by ANOVA using the mean of the three plant shoots from each pot at each harvest to compare differences between times and severity of defoliation.

RESULTS

Experiment 1

At both harvests there were no significant differences between simulated damage or weevil feeding treatments at any level of defoliation for any of the variables measured. Data were therefore combined for the remaining analyses. After 10 weeks the two most severe defoliation treatments had reduced shoot and root lengths by up to 50% and dry weights by up to 70% compared with untreated plants (Table 1). This pattern continued to the final harvest at 15 weeks, with significant reductions in root/shoot lengths and yields with increasing levels of defoliation. Root and shoot lengths were reduced by up to 30% and dry weights by over 60% respectively (Table 1).

Seedling survival at the first harvest was reduced from 100% to 81% for the most severe defoliation treatment. Few plants were lost between the first harvest at 10 weeks and the final harvest at 15 weeks.

TABLE 1: Length (cm) and dry weights (mg) of white clover shoots and roots harvested 10 and 15 weeks after defoliation treatment. Data combined for weevil feeding and simulated damage treatments.

Cotyledons removed	Length (cm)		Dry weight (mg)	
	shoots	roots	shoots	roots
10 weeks				
Untreated	5.5	4.6	5.0	2.5
1.0	4.6	4.7	4.0	1.8
1.5	3.2	3.2	1.8	1.3
2.0	2.6	2.1	1.4	0.8
SED	0.59	0.38	0.72	0.29
15 weeks				
Untreated	14.6	16.5	93.3	46.3
1.0	13.0	16.3	72.5	41.3
1.5	12.0	15.6	47.4	30.1
2.0	10.0	12.4	32.2	16.8
SED	0.86	1.36	8.6	5.1

Experiment 2

After 8 weeks the dry weight of plants defoliated after 1, 2, 4 and 8 days were more severely affected than defoliation at 16 days with yield reductions of about 40% (Table 2a). Those defoliated 16 days after cotyledon emergence were significantly reduced (by about 27%) compared with untreated seedlings but significantly less so than for the earlier treatments. By the final harvest at 15 weeks, all treated plant yields were similarly reduced compared with untreated plants by 20-30% although the trends noted at 8 weeks remained.

The effects of levels of defoliation were greater than timing, with decreasing dry weights corresponding with increasing defoliation. After 8 weeks, plants with 1, 1.5 or 2 cotyledon leaves removed were reduced in yield compared with untreated plants by 29, 50 and 72% respectively. After 15 weeks the equivalent yield reductions were 22, 36 and 53% respectively (Table 2b).

Survival rate of seedlings was generally high (over 93%) for most treatments with the highest losses (13%) occurring in plants defoliated 4 days after cotyledon emergence. Few plants were lost in the period between the first and second harvests.

TABLE 2: Dry weight (mg) of white clover plants harvested 8 and 15 weeks after (a) different times of defoliation and (b) different degrees of defoliation.

(a) Days between cotyledon emergence and defoliation						
	Untreated	1	2	4	8	16
						SED
8 weeks	13.1	8.2	7.3	7.7	8.1	9.7
15 weeks	127.5	91.1	88.3	88.9	91.0	101.3
						6.20

(b) Number of cotyledons removed					
	Untreated	1	1.5	2	SED
8 weeks	13.1	9.4	6.6	3.7	0.36
15 weeks	127.5	98.9	81.5	60.6	5.53

DISCUSSION AND CONCLUSIONS

The results from the comparison between real and simulated damage of white clover cotyledon seedlings in Experiment 1 suggest that the partial or complete defoliation of cotyledons by adult native weevils does not reduce subsequent plant growth to a greater or lesser extent than simulated defoliation. Thus weevil feeding damage simulated by clipping provides a representative measure of plant response to insect damage. An interval between cotyledon emergence and defoliation of 1-8 days appeared to have a similar effect on subsequent yields, with cotyledon damage after this time having a less severe effect. The cotyledon leaves presumably remain an important source of nutrient reserves for seedlings until the emergence of one or more true leaves, which then sustain plant growth.

The severity of defoliation influenced subsequent plant growth more than timing, especially the loss of 1.5 or both cotyledon leaves as noted in earlier work (Barratt 1980, 1985).

Little work has been reported on the effects of cotyledon defoliation with which to compare these results, and the comparability of simulated damage on more mature plants is questionable. However, Jackson (1980), using simulated damage, found that the yield losses in radishes were proportional to the amount of defoliation although this was not always the case, as compensation occurred in some instances. Skuhravy (1968) found that simulating the foliar feeding of the beetle *Leptinotarsa decemlineata* (Say) on potatoes could increase yields by up to 26%.

From the results of this work it is concluded that results from previous laboratory and field experiments where damage levels have been simulated by clipping, are validated. The results also show that insect damage occurring between germination and emergence of the true leaves has a similar effect on subsequent plant growth. In either cold or dry spring conditions that may occur in the field, seedlings often remain at this stage of growth for several weeks after germination. Insect feeding damage to individual plants can become increasingly severe over this period and it is this degree of defoliation which has been found to be of most consequence to plant growth.

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