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SCREENING WHITE CLOVER FOR GRASS GRUB RESISTANCE

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ABSTRACT

Breeding a white clover (*Trifolium repens* L.) with resistance to grass grub (*Costelytra zealandica* [White]) is a priority in New Zealand pastoral research. Controlled environment conditions giving high grass grub larval survival rates, were used to screen lines of white clover for resistance. The technique was successful in that differences in third instar larval weight gains were found, but the results were inconsistent. The relationship between white clover and grass grub growth appears to have a genetic component, but other factors complicate the relationship. White clover taproot diameter and leaf size did not correlate with grass grub larval weight gain.

KEYWORDS

Controlled environment, legumes, larval weight gain, root morphology, grass grub larvae.

INTRODUCTION

White clover (*Trifolium repens* L.) is the most important legume in New Zealand pastures, both as a source of nitrogen and as a component of high quality feed for animal growth. Grass grub (*Costelytra zealandica* [White]), a major pasture pest in New Zealand, preferentially attacks white clover, causing severe damage (East and Pottinger, 1984). The development of a white clover which is either resistant to grass grub and adversely affects larval growth, or tolerant to grass grub and grows well despite larval attack, would significantly improve pasture production in New Zealand.

While some legumes (*Lotus pedunculatus* Ca., *Medicago sativa* L., *Lupinus angustifolius* L.) resistant to grass grub have been identified (Farrell and Sweney 1972, 1974), their usefulness as replacements for white clover in pastures is limited (Kain *et al.* 1979). No resistant white clovers have been found. Wilson and Farrell (1979) reported variations in grass grub growth on different white clovers but were unable to produce consistent differences. Verry (1980, 1981) found no differences in grass grub growth and survival when comparing Huia with genotypes selected from grass grub prone areas. Wilson (1978a) suggested that at least one line of white clover existed which could tolerate grass grub attack.

The methods used in studying the interaction between grass grub and legumes have had some practical problems. These include poor plant growth due to excessive larval damage, disease (Wilson 1978a, 1978b), low larval survival (Wilson 1978a, 1978b, Wilson and Farrell 1979), high variability in larval survival (Verry 1980), extremes in soil moisture and temperature (Wilson and Farrell 1979) and larval combat (Verry 1980). The resultant high variability has made it difficult to attribute differences in larval growth on white clover to resistance.

Gaynor *et al.* (1985, 1986) developed a technique using controlled environment conditions to overcome some of the problems mentioned, when comparing larval growth on bean, lotus, and white clover. Their results suggested that there may be differences in larval growth on different white clover plants and that the technique should be sufficiently sensitive to detect any differences.

The experiments described in this paper were designed to screen a range of white clovers for differences in larval growth, and to determine whether variations in root morphology were correlated to larval growth.

MATERIALS AND METHODS

Experiment 1

This experiment was designed to screen a range of white clover lines for resistance to grass grub, using larval weight gain as the indicator. The populations screened were Grasslands Huia, Grasslands Tahora, G18, three white clover collections from grass grub-prone areas of Takapau,

Hawke's Bay, and Grasslands Maku (*Lotus pedunculatus* Cav.) as a resistant control. They were grown from seed in 10 cm diameter plastic pots containing Opiki peat loam with a base dressing of 3 g/litre dolomite lime and 2 g/litre superphosphate. Each pot was fitted with nylon mesh over the drain holes to prevent the larvae from escaping. The plants were inoculated with rhizobium strains NZP 560 (white clover) and NZP 2309 (lotus).

The plants were initially maintained in a glasshouse for three months. On 19 March 1984, 67 plants of each white clover line, plus 20 of lotus and 10 pots of soil-only were transferred to controlled environment rooms. These were maintained at 18 °C (±0.5 °C), 14-hour photoperiod with mean photosynthetically active radiation of 742-749 $\mu E/m^2$/second and 75% relative humidity. All pots were hand watered daily to maintain suitable moisture levels. The plants were trimmed to pot height before being transferred into the controlled environment rooms. Hoaglands solution was given to each plant on 30 March.

Third instar grass grub larvae were collected from the field in Nelson and air freighted to Palmerston North. The larvae were kept cool and moist and handled carefully; any obviously diseased larvae were discarded. Individual larvae were weighed, placed into a hole dug under each plant and covered with soil on 27 March. Only one larva was put in each pot. After 22 days the larvae were removed from the pots and reweighed to give a weight gain (or loss) measurement for each larva.

The larval weight gains were used as a basis for selecting two groups of white clover. One group contained plants which resulted in low larval weight gains, and the other high larval weight gains. G18 plants were excluded from selection.

Experiment 2

The two selected groups from the screening in Experiment 1 comprised 90 clovers with low larval weight gains (18.0-64.6 mg, mean = 43.7 mg), and 25 with high larval weight gains (75.6-111.1 mg, mean = 92.5 mg). The number of plants selected from each of the five clover lines are presented in Table 1.

The selected genotypes were divided on 18 January 1985 to give five copies of each, all of which were repotted. The plants were transferred to the controlled environment rooms on 26 March 1985, and the grass grub larvae were put into each pot 14 days later. The experimental conditions were the same as in Experiment 1. The larvae were removed and reweighed 22 days later. Hoaglands solution was not added as the plants were all growing vigorously.

Table 1. Number of plants selected from five white clover lines on the basis of low or high grass grub larval weight gains.

White clover line	Low weight gains	High weight gains
Huia	17	8
Tahora	18	6
Collection 1	13	6
Collection 2	20	2
Collection 3	22	3
Total	90	25

Experiment 3

This experiment was designed to examine the effect of white clover root morphology (i.e., mean root diameter) on grass grub growth. Since mean taproot diameter is positively correlated with leaf size (Caradus, 1977), the lines used were chosen on the basis of leaf size. Seed of G18, Dusi (two large-leaved cultivars), Huia, Menna (two medium-leaved cultivars), Kent Wild White (a small-leaved cultivar) and Collection 2 (a small-leaved ecotype) was sown on 16 January 1985. Seedlings were transplanted into pots containing Egmont loam soil with 1 g P/kg dry soil as 30% potassic superphosphate added. The Egmont loam soil is more friable than the Opiki peat loam, making it easier to remove and wash the clover roots. Forty-eight plants of each line (24 into each room) were put into the controlled environment rooms on 3 May, immediately after the completion of Experiment 2. The experimental conditions were the same as in Experiment 1. Grass grub larvae were put into each pot three days later. The larvae were removed and reweighed 23 days later. Hoaglands solution was not added. One day before larvae were removed, leaflet width measurements were made on two leaves per plant. After larvae were removed, the plants were separated into petioles plus leaves, stolons and roots. These were dried and weighed to give shoot and root dry weights. The taproot diameter of each plant was also measured.

RESULTS AND DISCUSSION

Experiment 1

In Experiment 1 larvae lost weight in soil-only and grew poorly on lotus, the resistant control, by comparison with all white clovers (Table 2). Larval weight gain on lotus was 35% of that on white clover. Grass grub larvae grew more on G18 than on the other white clover lines, suggesting that G18 is a more favourable host plant. There was no significant difference in larval growth between the other white clover lines. Taking Huia, the most widely grown white clover in New Zealand, as a standard non-resistant plant, none of the populations were resistant to grass grub. However, a wide range of larval growth on white clover was observed. On some plants, particularly in Collections 2 and 3 (Tables 1 and 2), larval growth was lower. This suggested that plants may be selected on the basis of low larval weight gains which could lead to developing white clovers resistant to grass grub. Therefore, plants with the lowest and highest larval weight gains were selected for comparison in Experiment 2. G18 plants were excluded from selection because they appeared to be a more favourable host relative to Huia, but the growth of larvae on G18 was further investigated in Experiment 3. The larval survival rate was 96%.

Table 2. Number and mean weight gain of third instar grass grub larvae on six white clover lines, lotus or soil for 22 days, Experiment 1, (from Gaynor *et al.* (1985)).

	Number of surviving larvae	Mean weight gain (mg)
White clovers		
G18	62	69.1
Collection 1	62	63.0
Tahora	66	62.4
Huia	66	61.9
Collection 2	66	59.6
Collection 3	63	59.3
LSD 5% for clover comparisons		5.8
Maku lotus	19	22.2
Soil	9	-15.3

Experiment 2

The two selections of plants from Experiment 1 (low and high larval weight gains) showed no difference (LSD 5%) in larval weight gains in Experiment 2 (Table 3). This indicates that selecting white clovers on the basis of larval weight gain in the initial screen has not produced significant differences between the two selected groups. The results suggest, however, that some progress may be possible with further screening. The large variability of the grass grub larval weight gains (Table 3), even on the five copies of each genotype, indicates the importance of having a large number of genotypes and/or copies of a genotype to produce a statistically significant difference.

The five lines were compared by combining the data for low and high selections; differences in larval growth were found. Larval weight gain on the plants selected from Huia was higher than on the other lines (Table 4). This contrasts with the results obtained in Experiment 1, in which Huia gave larval weight gains not significantly different from the other clovers. The difference between the two experiments may be the result of the disproportionate number of plants selected that gave low larval weight gains and high larval weight gains (Table 1). The larval survival rate was 88%.

Experiment 3

There was a high correlation ($r = 0.98^{***}$) between leaflet width and taproot diameter for all lines. The large-leaved cultivars (G18 and Dusi), had thicker taproots, and higher root dry weights and shoot dry weights (Caradus, unpub. data). The other four lines had similar root and shoot dry weights.

Lines with large, medium, and small leaves, and associated variations in root morphology (e.g., taproot diameter), gave no difference in mean larval weight gains (Table 5). This contrasts with the results obtained in

Table 3. Number of white clover genotypes and surviving larvae, and mean weight gain of third instar grass grub larvae over 22 days, on low and high larval weight gain selections, Experiment 2, (five copies of each genotype).

Selection category	Number of genotypes	Number of surviving larvae	Mean weight gain (mg)
Low	90	398	37.2
High	25	106	40.2
Total	115		
LSD 5%			3.7
Range of genotype mean larval weight gains			16.4-65.9
LSD 5%			22.3

Table 4. Number and mean weight gain of third instar grass grub larvae grown for 22 days on larval weight gain selected genotypes from five white clover lines from Experiment 1 (low and high weight gain combined) — Experiment 2, (five copies of each genotype).

	Number of genotypes	Number of surviving larvae	Mean weight gain (mg)
White clover line [1]			
Collection 1	19	79	37.4
Tahora	24	108	35.2
Huia	25	113	44.6
Collection 2	22	98	33.7
Collection 3	25	106	37.6
Total	115		
LSD 5%			4.9

[1] These lines are selections from Experiment 1.

Table 5. Number and mean weight gain of third instar grass grub larvae on six white clover lines for 23 days, Experiment 3.

	Number of surviving larvae	Mean weight gain (mg)
White clover line		
G18	44	24.8
Dusi	45	24.5
Huia	44	29.9
Menna	46	22.8
Collection 2	44	23.9
Kent Wild White	45	21.9
p		n.s
LSD 5%		8.1

Experiment 1, in which larval growth was greater on G18 than on Huia and Collection 2 (and Experiment 2, where larval growth was greater on Huia than on Collection 2). Experimental results may have been affected by an interaction between the different soil types and the rate of plant and/or larval growth. More work is being undertaken to determine this, but results indicate that the relationship between white clover and grass grub growth is not a simple genetic one. The larval survival rate was 93%.

CONCLUSION

Grass grub larval growth on different white clovers in a controlled environment has successfully been compared. Differences in larval growth between white clover lines have been detected, but the results have been inconsistent. This suggests that the relationship between grass grub growth and white clover is a complex of genetic and environmental factors. Selecting white clovers on the basis of larval weight gain has not been entirely successful in the initial screen. However, the results indicate that further selection using this technique may produce significant differences.

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SYMPOSIUM DISCUSSION

Dr B.T. Hawthorne, Plant Diseases Division, DSIR
Are there ecotypes of grass grubs, and was the source of grass grubs the same in each experiment?

van den Bosch
The grass grubs were all collected from the same area in Nelson.

Dr O.R.W. Sutherland, Entomology Division, DSIR
We do not really know whether there are ecotypes of grass grubs. The grubs for all experiments were

collected in the same 2 or 3 fields on the same farm in an attempt to reduce genetic variability.

Hawthorne

So it is feasible that grass grub ecotypes could be a source of considerable variation.

van den Bosch

By collecting them from the same area we tried to reduce that as much as possible. The results indicate that most of the variability in this experiment is from the grass grubs, rather than the plants.

Dr S.D. Carson, Forest Research Institute

Would it be better to use four or five grass grubs/pot rather than just one?

Bosch

That is an alternative we may look at in the future. It has been done in the past and there is a question as to whether the interaction between the larvae overrides any biomass accumulation.

Dr H.S. Easton, Grasslands Division, DSIR

In Experiment 2 comparing the high and low selected progeny, and in Experiment 3 comparing Huia and the collection of data, you concluded that there was no significant difference. I wonder if you are being too conservative in your inference because you were not a long way from significance, and they were in the right direction. I think you should try the 10% significance level.

Dr H.A. Eagles, Plant Physiolgy Division

Considering that there is genetic variation present, and the magnitude of the problem of grass grub in white clover, would it be reasonable to go into a large recurrent selection programme using your screening technique to obtain grass grub resistant cultivars?

van den Bosch

That would be one of the aims if we can definitely identify resistance sources. At this stage, because of the inconsistencies, we would not be positive that there is a resistance factor there, or that we have resistant plants.

Eagles

Do you need to have absolute resistance?

van den Bosch

We have retained the plants from Experiment 2 so we could take plants which were too low in both the first and second experiments and cross them and carry on in that direction. But we are first working out if this technique is good enough to be definite.

Eagles

Your numbers are very small for this sort of problem compared for example with the numbers that lucerne breeders work with to get resistance to aphids.

van den Bosch

That is one of the limitations to this technique. Numbers above 300-400 are quite unmanageable.

Dr S.D. Carson, Forest Research Institute

Why do you separate resistance and tolerance?

van den Bosch

The ideal would be to have a plant which was both resistant and tolerant, but we have to be realistic and accept that we will probably never obtain that. The degree of resistance will probably never be comparable with *Lotus*. At present we are looking at one factor — tolerance has been looked at in the past, we are only looking at resistance.