

LABORATORY INVESTIGATIONS INTO FEEDING PREFERENCES OF ADULT *SITONA LEPIDUS* GYLLENHAL

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ABSTRACT

Measurements of feeding damage by adult clover root weevil (*Sitona lepidus*) on both seedlings and excised leaf material from thirteen pasture legume species were made in four laboratory experiments. Clover root weevil showed no feeding preferences in the choice and non-choice feeding experiments using seedlings. However, in both choice and non-choice experiments using excised leaf material from mature plants, food intake was higher on perennial *Trifolium* spp. compared to annual *Trifolium* spp. Feeding on leaf material from mature *Medicago* spp. and *Lotus* spp. was very limited in both choice and non-choice experiments.

Keywords: *Sitona lepidus*, feeding preferences, *Trifolium* spp., *Lotus* spp., *Medicago* spp.

INTRODUCTION

Clover root weevil, *Sitona lepidus* Gyllenhal was first identified in New Zealand pastures in the Waikato in 1996 (Barratt *et al.* 1996). A 1996 survey showed clover root weevil to be widespread over two infection loci, one in the Auckland area, the other in the Waikato/Bay of Plenty area (Barker *et al.* 1996). Clover root weevil is spreading rapidly outward from these two infestation centres at a mean rate of 35 km per year (Willoughby and Addison 1997). The species is of European origin and is found as far north as 66-67° N in Finland (Markkula and Koppa 1960). Therefore, it is likely the weevil could colonise the whole of New Zealand.

Weevils of the genus *Sitona* have been identified as one of the most common pests of pasture legumes in Europe (Murray 1991). The adult weevils are known to cause extensive damage to the leaves of seedlings (Murray and Clements 1992) and mature white clover plants (Mowatt and Shakeel 1988). While adult clover root weevil feeds on a large number of legume species, it has previously been shown to exhibit strong feeding preferences for white clover (*Trifolium repens*), strawberry clover (*T. fragiferum*) and alsike clover (*T. hybridum*) (Murray 1996a). Of these three species, white clover is widely used as the companion species to ryegrass (*Lolium perenne*) while alsike and strawberry clover occur to a limited extent in New Zealand pasture.

The larvae of *Sitona* spp. are the most damaging stage of the life cycle. First instar larvae feed on the root nodules of clover plants, while larger larvae feed on progressively larger roots (Byers and Kendall 1982). Larval feeding can cause significant damage to clover plants as a result of direct damage and by facilitating penetration of fungal disease via the feeding scars (Newton and Graham 1960).

Because New Zealand's pastoral agriculture depends heavily on legumes to fix atmospheric nitrogen, it is important to identify legume cultivars or species that are resistant or tolerant to clover root weevil feeding. This paper reports on four experiments investigating feeding preferences of adult clover root weevil on both seedlings and excised mature leaf material of 13 pastoral legume species.

MATERIALS AND METHOD

Adult clover root weevil were collected by suction sampling from pasture as described in Willoughby and Addison (1997) and then kept in a refrigerator at 5°C

until required. Prior to the start of each experiment, the weevils were removed from the refrigerator and starved for 24 hours.

The legumes used in the experiments are listed in Table 1. The plants were raised from seed, in Horotiu silt loam, in a screen house exposed to ambient temperatures between 5°C and 35°C. The plant species were chosen to cover a range of legumes which are currently used or could potentially be used in New Zealand's pastoral grazing systems.

Experiment 1: non-choice feeding tests (excised plant material)

Single leaves from plant material of similar physiological age and morphology were excised from plants and within 15 minutes placed on a moistened filter paper in a 90 mm diameter plastic petri-dish, together with one adult weevil. Each treatment (plant species) was replicated ten times. The dishes were kept at 18°C in a 16:8 light:dark regime. After 48 h the weevils were removed and, by placing a transparent grid divided into mm² over the remains of the leaf, the area consumed was estimated. To calculate the fresh weight of leaf material consumed, ten 50 mm² sections of leaf were removed from each legume species and weighed immediately.

Experiment 2: choice feeding tests (excised plant material)

A single leaf from each plant species was arranged randomly at equidistant spacing on moist filter paper in each of ten trays (270 mm x 160 x 5 mm high). Each tray was then placed in a plastic bag together with five weevils. The bag was then sealed to prevent weevil escape. The trays were kept at 18°C in a 16:8 light:dark regime. After 48 h, the weevils were removed and the area and weight of leaf material consumed was estimated as above.

Experiment 3: non-choice seedling feeding tests

Seeds of each legume species were sown into 180 ml pots half-filled with Horotiu silt loam. Ten replicates of each of the thirteen legume species were arranged in a randomised complete block design. When the cotyledon leaves of all seedlings had been present for at least 2 days (11 days after planting), the seedlings were thinned to a single seedling/pot and two weevil adults confined in each pot with a mesh cover. Damage to the seedlings was assessed after 6 and 12 h. Seedling damage was scored on a 0-4 scale; 0 = undamaged, 1 = minor damage to cotyledons, 2 = one cotyledon removed 3 = 1.5 cotyledons removed and 4 = two cotyledons removed.

Experiment 4: choice seedling feeding tests

One seed of each legume species were sown into pots (170 x 170 x 80 mm) containing Horotiu silt loam at equidistant spacing and 20 mm from the edge of the pot and grown to the cotyledon stage. Five weevil adults were then placed in the middle of the pot and confined with a mesh cover. Damage to seedlings was then assessed after 6 and 12 h as above.

The results of all four experiments were subjected to an ANOVA after the data were square root transformed. All results are reported as untransformed means.

RESULTS

Choice and non-choice excised leaf experiments

In the non-choice tests feeding on *Trifolium* spp. (with the exception of *T. dubium*) was significantly greater than on the other legume species tested ($P < 0.01$) (Table 1). Within the *Trifolium* spp., perennial *Trifolium* spp. (*T. repens*, *T. fragiferum*, *T. ambiguum* and *T. hybridum*) were consumed more rapidly than annual or biennial spp. (*T. pratense*, *T. subterraneum* and *T. incarnatum*) ($P < 0.05$). In the choice test, a similar pattern of feeding preference was found, although in this case feeding was observed only on *Trifolium* spp.

Choice and non-choice seedling experiments

There were no differences in feeding damage to the cotyledon stage seedlings of different legume species with mean feeding scores of 3.4 (± 0.23) and 2.5 (± 0.35) in the choice and non-choice feeding tests respectively, 6 hours after weevils were introduced. After a further 6 h all seedlings were totally defoliated in both the non-choice and choice feeding tests.

TABLE 1: Food intake of adult *S. lepidus* fed a range of legume species in choice and non-choice feeding tests.

Plant species	Non-choice mg/weevil/h	Choice mg/weevil/h
<i>Trifolium repens</i> (cv. Grasslands Pitau)	0.317	0.119
<i>T. fragiferum</i> (wild type selection)	0.298	0.122
<i>T. ambiguum</i> (cv. Endura)	0.242	0.100
<i>T. hybridum</i> (wild type selection)	0.245	0.111
<i>T. pratense</i> (cv. Grasslands Pawera)	0.162	0.026
<i>T. subterraneum</i> (cv. Karridale)	0.121	0.025
<i>T. incarnatum</i> (wild type selection)	0.159	0.001
<i>T. dubium</i> (wild type selection)	0.002	0.0
<i>Medicago sativa</i> (cv. Grasslands Oranga)	0.006	0.0
<i>M. lupulina</i> (wild type selection)	0.008	0.0
<i>M. arborea</i> (wild type selection)	0.019	0.0
<i>Lotus pedunculatus</i> (cv. Grasslands Sunrise)	0.020	0.0
<i>L. corniculatus</i> (cv. Grasslands Goldie)	0.018	0.0
LSD _{0.05}	0.076	0.022

DISCUSSION

The experiments on excised mature plant material gave similar clover root weevil feeding preference results as previous studies (Murray 1996a; Murray and Clements 1994) which identified that the preferred host range of clover root weevil was largely restricted to *Trifolium* spp.

The seedling damage scores observed in the seedling experiments 6 h after weevil addition related to the removal of between 1.5-2 cotyledons from each seedling. Previous work by Barratt and Jones (1994) on cotyledon removal from white clover seedlings, indicated that this extent of cotyledon loss resulted in reductions in shoot and root length by 30%, dry weights by over 60% and seedling survival from 100% to 81%.

One of the strategies suggested to reduce possible effects of clover root weevil on the legume component of pastures is the use of plant material which is tolerant or resistant to clover root weevil. The results from the mature detached leaf material experiments suggest that this may be possible. However, the mechanisms that trigger feeding preference have not been identified. The results of these experiments suggest that the physical makeup of the plant may be partly responsible for feeding preferences in clover root weevil. In general, the perennial *Trifolium* spp. used in this study were hairless, while the annual and biennial *Trifolium* spp., *Medicago* spp. and *Lotus* spp. were hairy, with the exception of lucerne (*M. sativa*) which had smooth leaf surfaces.

While it is possible that the physical structure of the plant may be important in deterring clover root weevil feeding, the chemical composition of plants also plays an important role. (Akeson *et al.* 1970) found that some water soluble carbohydrates, particularly some sugars, stimulated the feeding activity of *Sitona cylindricollis* and they concluded that small differences in chemical composition could cause large differences in feeding response. Murray (1996a) suggested that very short survival times of clover root weevil feeding on *T. dubium* were due to toxicity rather than an antifeedant starvation effect, which appeared to be the case on many of the other plant species offered to the weevil. Differing amounts of feeding by *Sitona* spp. on different varieties of white clover have also been related to the cyanogenic potential of the white clover variety (Murray 1996b). Age of the plant also affects the concentrations of secondary substances which may act as phagostimulants, attractants or repellents (Crawley 1983).

In conclusion, the identification and exploitation of resistance mechanisms involved in the feeding preference of *S. lepidus* could be important in developing

resistant leguminous material for pastures in New Zealand. However, the preliminary results from the seedling choice experiments suggests that further research needs to be carried out to determine at what age plants become unattractive to adult *S. lepidus*.

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