EFFECTS OF WHITEFRINGED WEEVIL LARVAL FEEDING ON RYEGRASS AND WHITE CLOVER IN THE LABORATORY

S. HARDWICK and R.A. PRESTIDGE

AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton

ABSTRACT

The feeding behaviour of whitefringed weevil (*Naupactus leucoloma*) larvae was investigated using a slant board technique. First instar larvae are a non-feeding stage. Instars 2-4 started feeding immediately on clover roots but did not feed on ryegrass until day 8. Individual larvae of instars 8-11 reduced clover root weight by 81%, total clover weight by 40%, and reduced the nitrogen fixation rate of clover plants by 92%. Larvae feeding on white clover increased in weight by 5% (2-4 instars) and 3% (8-11 instars). On ryegrass larval weight decreased by 8% (2-4 instars) and 14% (8-11 instars).

Keywords: Whitefringed weevil, *Naupactus leucoloma*, white clover, ryegrass, slant board

INTRODUCTION

Whitefringed weevil (*Naupactus leucoloma* (Boheman)) (WFW), recently transferred from the genus *Graphognathus* by Lanteri and Marvaldi (1995), is a parthenogenetic weevil native to South America. It was first reported in New Zealand in 1945 (Todd 1968) and is known from lowland pastures of the North Island and north-eastern parts of the South Island. In New Zealand, reproductively mature adults are present from late November to mid April. Recruitment to the larval stage is maximised in March (Hardwick and Prestidge 1994). Adult WFW feed on the foliage of pasture plants but are not considered to be economically damaging (Todd 1968). The larval stages feed on roots and are considered of greater pest potential. East (1982) demonstrated that productivity in pure stands of white clover (*Trifolium repens* L.) was reduced by up to 28%, and mixed swards of perennial ryegrass (*Lolium perenne* L.) and white clover by up to 10% when populations of 234-722 larvae /m² were present. Larvae that feed on plant roots present problems in direct observation of feeding behaviour. Byers and Kendall (1982) and Murrery and Clemants (1992) adopted the slant board technique developed by Kendall and Leath (1974), for direct observation of feeding by *Sitona* larvae on legume root systems. This paper describes the results of three experiments in which the feeding behaviour of three different WFW larval instar groupings was assessed using a slant board technique.

MATERIALS AND METHODS

Plant preparation

White clover cv 'Grasslands Pitau' and perennial ryegrass cv 'Yatsyn' 1 were grown individually from seed in pots of coarse river sand. To provide for white clover nodulation, a suspension of *Rhizobium* bacteria was watered into each pot when the seedlings were at the first true leaf stage. When the clover seedlings had reached the third true leaf stage and the ryegrass plants had 2-3 tillers, they were removed from the pots and washed under running water to remove sand from the roots. These seedlings were then transferred to the slant boards where they were allowed to stabilise for fourteen days prior to the larvae being added.

Slant board design

Each slant board was assembled on a plastic tray 275 x 170mm, and comprised a plant or plants placed between sheets of finely woven 100% polyester cloth which were in turn sandwiched between sheets of capillary matting. The entire assembly was

then held in place with a sheet of glass that fitted inside the plastic tray, leaving 5mm gaps at the bottom and top, and fastened with a paper binder clip. The plants were placed in this assembly so that the roots were housed entirely between the polyester cloth sheets with the foliage projecting from the top. Each slant board stood in a section of plastic guttering and watered via a drip irrigation system to maintain 20mm of water in the guttering at all times to allow the uptake of water by the capillary matting. Eighty slant boards were arranged in 8 blocks of ten, with each block contained in a separate length of guttering. During all experiments the slant boards were kept in a controlled environment room at 18 °C under a photoperiod of 16 hours light: 8 hours dark.

Larval treatments

First instar larvae were used in Experiment 1, while Experiment 2 was set up with 2nd-4th instar larvae reared from eggs in a laboratory colony, (Gough and Brown 1991). Eighth to eleventh instar larvae were collected from the field for Experiment 3. Larvae were assigned to instar groups by measuring their head capsules at 50 x magnification with a graduated eyepiece and comparing the measurements to the criteria in Gough and Brown (1991).

Experimental design

Three experiments were carried out using plants grown from the same seed line. In each of the experiments there were three different plant treatments: i) a single white clover plant, ii) a single ryegrass plant, and iii) 1 white clover and 1 ryegrass plant per slant board. A single larva of the appropriate instar was added to the slant board except for untreated plants. To maximise the number of replicates used, each block received 1 replicate of each treatment and the remaining four slants in the block received four randomly selected treatments so that eighty slant boards were incorporated into a randomised incomplete block experimental design. The WFW larvae were allowed to feed for fourteen days before the experiments were terminated. Each slant board was opened every 2nd day with the position of the WFW and any visible root damage being noted. The nitrogen fixation activity of each clover plant was measured within 24 hours of termination of the experiment using the acteylene reduction assay similar to that outlined in Wardle *et al.* (1994). The plants were dissected into roots and shoots, dried at 100 °C, and weighed for yield estimates. Larval weights were recorded before and after each experiment.

The results were analysed with Genstat using REML (Patterson and Thompson 1971).

RESULTS

In all three experiments mortality among WFW larvae averaged 1.2%. All control plants grew well and appeared healthy for the duration of the experiments, with no mortality due to diseases or other disorders

Experiment 1

There was no observable feeding by 1st instar WFW larvae (n=37) which stayed stationary and appeared not to be associated with any plant roots during the fourteen days of the experiment. Three larvae which had moulted to the 2nd instar were observed feeding on the roots of white clover. Feeding by these individuals consisted of chewing on root hairs and in one case, burrowing into a branch root. There were no significant differences ($P>0.05$) in clover (193.4 mg) and ryegrass (132.1 mg) dry weight and nitrogen fixation (29.4 ml/h/plant), between the plants with or without 1st instar WFW larvae.

Experiment 2

All 2-4 instar WFW larvae had moved onto the clover roots in both the 'white clover' only and mixed 'white clover, ryegrass' treatments by day 2. Feeding consisted of the destruction of root hairs, detachment of fine roots and scarring of larger roots. For the 'ryegrass' only treatment, larvae did not move onto the roots until day 8 where feeding was similar to that on white clover but less extensive. Larval weight change was significantly affected by diet ($P<0.01$). WFW larvae feeding in the 'white clover only' treatment increased their weight by 5%, while the weight of larvae in the 'ryegrass only' treatment decreased by 8%. Larval weight in the mixed 'white

clover plus ryegrass' treatment remained unchanged. There were no significant differences ($P>0.05$) in clover (205 mg) and ryegrass (108.5 mg) dry weights and nitrogen fixation (26.2 ml/h/plant), between control and larval infested plants at the termination of the experiment.

Experiment 3

There was extensive feeding by the 8-11 instar larvae on white clover in both the 'white clover' only and mixed 'white clover-ryegrass' treatments (Table 1). In the 'white clover-ryegrass' treatment larvae initially fed on the white clover, but on day 8, the majority of larvae ($n=9$) were observed feeding on ryegrass roots. On clover the feeding was characterised by the severing of small roots and main root branches. In 4 out of 14 replicates the clover plants died, observations suggesting that further plant losses would have occurred had the experiment not been terminated at day 14. Larval feeding caused a significant ($P<0.001$) reduction in clover root dry weights from 92-95 mg to 16-19 mg. Shoot dry weight of the white clover was not significantly ($P>0.05$) affected by larvae during the experimental period. However, total clover dry matter per plant in slant boards containing a larva (115-132 mg) was significantly lower than that of the control clover plants (207-209 mg) ($P<0.01$). Nitrogen fixation rates were significantly ($P<0.001$) reduced from 31-26 ml per hour in control clovers to 2 ml per hour for plants subjected to larval feeding. In the 'ryegrass only' slant boards, plants suffered little feeding damage on the roots. The presence of larvae did not affect the dry weight of roots ($P>0.05$) or shoots ($P>0.05$). The presence of ryegrass had no significant effect ($P>0.05$) on clover root, shoot and hence total dry matter production.

TABLE 1: White clover and ryegrass dry weights (mg DM/plant) and nitrogen fixation (μ l/h/plant) of plants grown in the presence/absence of WFW larvae (8-11 instars).

Plant	Larvae $\pm$	Clover root	Total clover	Ryegrass root	Total ryegrass	N fixation rate ¹
Ryegrass	-	-	-	61.3	125.5	-
	+	-	-	47.2	103.7	-
Clover	-	95.5a	209.0a	-	-	26.4a
	+	18.7b	115.0b	-	-	2.6b
Clover +	-	92.2a	207.1a	49.8	96.4	31.4a
Ryegrass	+	16.6b	132.8b	41.2	108.7	2.2b
Significance		***	**			***

¹ Nitrogen fixation rate data analysed by log transformed rank data analysed using REML. Values followed by the same letter not significantly different. ** Significant at $P<0.01$, *** at $P<0.001$.

Larval weight change was significantly affected by plant species ($P<0.001$). Larvae in the 'white clover only' treatment increased their weight 3%, while the weight of those in the 'ryegrass only' treatment decreased 14%. Larval weight in the 'mixed white clover-ryegrass' treatment was similar at the start and termination of the experiment.

DISCUSSION

The absence of feeding amongst 1st instar WFW observed in Experiment 1 has been reported by Gough and Brown (1991), who also observed that the gut of first instar WFW larvae was not fully developed and contained a yolk-like substance. The weight gains by larvae in treatments containing 'clover only' in Experiments 2 and 3,

indicate that white clover is a more suitable food source for WFW than ryegrass, where weight losses occurred due to lack of larval feeding on ryegrass. In the 'ryegrass white clover' treatments, the lack of weight gain was due to larvae ceasing feeding on white clover around day 7 and then resuming feeding on ryegrass around day 8. This may have meant that a net weight gain during the first half of the experiments could have been cancelled out by a net weight loss in the latter half of the experiments. The switch in feeding may have been due to a change in quality of the detached white clover roots.

The absence of an effect on plant performance in Experiment 2 was due to the short period that plants were exposed to the larvae. It may take longer for damage due to early instar WFW feeding to become apparent. Hardwick and Prestidge (1994) reported that in northern pastures in New Zealand, instars 2-7 can be present at 100-200 larvae/m² in the March-May period. In Experiments 2 and 3, the plants were only exposed to a single larva. In the field this situation would be unlikely as WFW eggs are laid in gelatinous masses of up to 50 eggs causing a clumped distribution of early instars (Hardwick unpublished) with almost certainly more than one larva feeding on the roots of individual plants.

The most severe root damage came from the 8-11 instar group which severed the larger roots causing major loss of root material, and plant mortality. This type of feeding has been observed in late instar larvae of *Sitona discoides* on lucerne (*Medicago sativa* L.) by Aeschlimann (1986). Murray and Clements (1992) concluded that the severing of roots was the feeding method that accounted for many of the losses in white clover plants that were attacked by *Sitona* weevils. If the clover plant survived such a drastic root pruning in the field, there is strong evidence to suggest that its productivity would be affected for over three months after the initial damage (Davidson 1978).

Severe larval damage to white clover roots caused a 92% reduction in nitrogen fixation rate, which in field conditions would cause white clover to become less competitive than ryegrass and eventually lead to declining pasture productivity. To maintain productivity in pastures with declining populations of white clover, alternative sources of nitrogen would have to be applied leading to increased farm costs.

Results suggested that WFW larvae actively select white clover, so that pastures with high WFW larval populations may suffer a decline in white clover content over a period of several years. Studies by King and East (1978) show that in mixed white clover and ryegrass pastures, WFW numbers rose over a period of 1-2 years when white clover content was at its highest and that as the percentage of grass increased, WFW larval populations decreased.

CONCLUSIONS

Late instar WFW reduce white clover production, nitrogen fixation and may cause white clover mortality. The larvae also display a preference for white clover over ryegrass. However, studies over a longer period of time using a slant board technique are required to establish the relationship between early instar WFW and white clover.

ACKNOWLEDGEMENTS

We thank Bruce Willoughby for technical assistance and advice, and Dr Neil Cox for statistical analysis.

REFERENCES

- Aeschlimann, J.-P., 1986. Rearing and larval development of *Sitona* spp. (Coleoptera, Curculionidae) on the root system of *Medicago* spp. plants (Leguminosae). *J. Appl. Ent.* 101: 461-469.
- Byers, R. A. and Kendall, W. A., 1982. Effects of plant genotypes and root nodulation on growth and survival of *Sitona* spp. larvae. *Env. Ento.* 11: 440-443.
- Davidson, R. L., 1978. Effects of root feeding on foliage yield. *Proc. 2nd Aust. Conf. Grassl. Invert. Ecol.*: 117-120.
- East, R., 1982. Interactions between whitefringed weevil *Graphognathus leucoloma* and legume species in the northern North Island. *N.Z. J. Agr. Res.* 25: 131-140.

- Gough, N. and Brown, J. D., 1991. Development of larvae of the weevil, *Graphognathus leucomela* (Coleoptera: Curculionidae) in Northern Queensland. *Bull. Ent. Res.* 81: 385-393.
- Hardwick, S. and Prestidge, R.A., 1994. Phenology of whitefringed weevil (*Graphognathus leucomela*) in pasture in northern New Zealand. *Proc. 47th N.Z. Plant Prot. Conf.*: 257-260.
- Kendall, W.A. and Leath, K.T., 1974. Slant board culture methods for root observations of red clover. *Crop Sci.* 14: 317-320.
- King, P. D., and East, R., 1978. Effects of pasture composition on the dynamics of *Heteronychus arator* and *Graphognathus leucomela* populations (Coleoptera: Scarabaeidae and Curculionidae). *Proc. 2nd Aust. Conf. Grassl. Invert. Ecol.*: 79-82.
- Lanteri, A.A., and Marvaldi, A.E., 1995. *Graphognathus* Buchanan a new synonym of *Naupactus* Dejean, and systematics of the *N. leucomela* species group (Coleoptera: Curculionidae). *Coleopt. Bull.* 49: 206-228.
- Murray, P.J. and Clements, R.O., 1992. A technique for assessing damage to roots of white clover caused by root feeding insects. *Ann. Appl. Biol.* 121: 715-719.
- Patterson, H.D. and Thompson, R., 1971. REML Recovery of inter-block information when block size is unequal. *Biometrika* 58: 545-554.
- Wardle, D.A., Nicholson, K.S., Ahmed, M. and Rahman, A., 1994. Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L. *Plant and Soil* 163: 287-297.
- Todd, D.H., 1968. Laboratory evaluation of insecticides against whitefringed weevil (*Graphognathus leucomela* (Boheman) (Coleoptera: Curculionidae)). *N.Z. J. Agric. Res.* 11: 903-910.