

**SILENE VULGARIS: A NEW GRASS GRUB RESISTANT PLANT**O.R.W. SUTHERLAND,<sup>1</sup> J.J. DYMOCK,<sup>1</sup> G.A. LANE,<sup>2</sup>  
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The development of new techniques for introducing foreign genes into white clover (*Trifolium repens*) (White and Greenwood 1987) has given recent impetus to the search for genes which may confer pest resistance. Over recent years grass grub (*Costelytra zealandica*) resistance in a number of plants has been studied (Sutherland *et al* 1982), and the biochemical mechanisms of resistance elucidated (Biggs *et al* 1986). Another plant, *Silene vulgaris*, has recently been found to be highly resistant to the European white grub *Melolontha melolontha* (T. Wildbolz pers. comm.). Known as the bladder campion, *S. vulgaris* is a widely distributed perennial in Europe, found in cultivated land, grassland and wastes. It was first recorded in New Zealand in 1871 and is found on Great Barrier Island, Waikato and much of the South Island (Garnock-Jones 1981).

**Laboratory screening of *Silene vulgaris***

Larval growth on *S. vulgaris* was compared with that on *Trifolium repens* and *Lotus pedunculatus* as susceptible and resistant controls respectively, under controlled climate conditions (Dymock *et al* 1989). Field collected larvae were weighed and a single larva was buried under each of 24 individually potted replicates of each species. The larvae were collected and reweighed after 22 days. The weight gains were analysed with initial weight as a covariant and LS means were used to test for significant differences at the 5% level.

**Feeding deterrent bioassay**

Field collected third instar grass grub larvae, starved for 24 h were enclosed individually in petri dishes each with a 1.5 cm agar/cellulose powder disc containing feeding stimulant and either ethanolic extracts of *S. vulgaris* (at a concentration of 0.8 g fresh root weight/ml) or distilled water as control. The feeding deterrent activity was assessed by counting the number of faecal pellets produced in 24 h and is expressed as the concentration of extract reducing feeding by 50% (FD<sub>50</sub>), with error limits (FD<sub>50</sub> ± c, FD<sub>50</sub> × c) (See Lane *et al* 1985 for details).

**Toxicity bioassay**

This was performed as previously described (Hutchins *et al* 1984). Starved field-collected third instar larvae were dosed orally with 10 µl of test root extract and thereof in aqueous solution. All materials were tested at a concentration of 10 g fresh weight of root/ml. Larvae were inspected after 24 h and dead and moribund (alive but not responsive to physical stimulation) larvae were recorded. Control insects were treated identically and dosed with equivalent volumes of distilled water.

**Extraction of *Silene vulgaris* root**

The washed roots (580 g fresh weight) of pot-grown *S. vulgaris* were extracted with 95% EtOH (2 litres) in a Waring blender and the mixture filtered. Aliquots of the filtrate were tested for activity. The filtrate was evaporated to dryness and absorbed onto reversed-phase silica gel (20 g) and packed onto a column containing further Rp silica gel (200 g). The column was eluted with water and water-methanol mixtures of increasing methanol concentration, collecting 100 ml fractions. Aliquots of each fraction were tested at concentrations equivalent to 0.8 gfw/ml of root for feeding deterrent activity and 10 gfw/ml of root for toxicity.

**Results**

The weight gains of grass grub larvae in the pot trials are shown in Table 1. The weight gain of larvae grown on *Silene vulgaris* was significantly lower than that of larvae grown on white clover but was not as low as that of larvae offered *L. pedunculatus*.

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**TABLE 1: Weight gain of third instar grass grub larvae on *Silene vulgaris*, *Trifolium repens* and *Lotus pedunculatus* (N = 25).**

Species	Larval weight gain $\pm$ SE (mg)	No. surviving
White clover	54.8 $\pm$ 4.7	19
<i>Silene vulgaris</i>	22.6 $\pm$ 4.9	18
<i>Lotus pedunculatus</i>	4.7 $\pm$ 4.4	23

**TABLE 2: The toxicity of extracts of root of *Silene vulgaris* to third instar grass grub larvae (% dead/moribund larvae) (N = 20).**

Species	Concentration (gfw/ml)	
	1	10
<i>S. vulgaris</i>	5	85
<i>L. pedunculatus</i>	—	55
Control	0	0

The feeding deterrent activity of *S. vulgaris* root extracts was determined at several concentrations to give an  $FD_{50}$  of 0.4 gfw/ml (c.2.5). Extracts of *L. pedunculatus* root tested simultaneously gave an  $FD_{50}$  of 11.1 gfw/ml (c. 1.4). Thus, although *S. vulgaris* did not appear in the pot trials to be as resistant as lotus it nevertheless had greater feeding deterrent activity in the laboratory assays. The toxicity of the *S. vulgaris* extracts, by oral dosing, was also greater than for *L. pedunculatus* (Table 2).

When the ethanolic extract of *S. vulgaris* was separated into fractions by reversed-phase chromatography all fractions showed feeding deterrent activity at 10 gfw/ml (Table 3) but the aqueous fraction, water/methanol (2/3) fraction and water/methanol (1/4) fraction all showed particularly high activity. The aqueous fraction and the water/methanol, 3/2 fraction also showed high toxicity. A crystalline material was obtained from the aqueous fraction which on preliminary evidence, appears to be an oxalate salt. At 0.8 mg/ml this gave a 61% reduction in feeding and at 15 mg/ml a 10  $\mu$ l dose affected 75% of the larvae.

In conclusion, *Silene vulgaris* was shown to be resistant to grass grub larvae and has demonstrable and significant feeding deterrent activity and toxicity. The root extracts can be fractionated to give several active fractions but the presence of a high concentration of oxalate appears to contribute to the observed activity.

**TABLE 3: Feeding deterrent activity and toxicity to grass grub larvae of *Silene vulgaris* root fractions (N = 20).**

Fraction from RP chromatography	% larval feeding	% dead/moribund larvae
Control	100	0
H <sub>2</sub> O	12	85
H <sub>2</sub> O/MeOH, 4/1	33	5
H <sub>2</sub> O/MeOH, 3/2	37	80
H <sub>2</sub> O/MeOH, 1/1	36	10
H <sub>2</sub> O/MeOH, 2/3	14	50
H <sub>2</sub> O/MeOH, 1/4	—	10
MeOH	21	0

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