A NEW TOXIN FOR GRASS GRUB AND BLACK BEETLE IN RESISTANT LOTUS MAJOR

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Summary

Crude aqueous extracts of lotus major (*Lotus pedunculatus*) are highly toxic to grass grub and black beetle larvae. The activity of extracts of two other *Lotus* species was measured and was found to correspond with the lower level of resistance of these species to grass grub. A novel technique for introducing material directly into the gut of the larvae is described.

INTRODUCTION

The resistance of lotus major (*Lotus pedunculatus*) to grass grub (*Costelytra zealandica*) and to black beetle (*Heteronychus arator*) is well-established (Farrell and Sweney 1972, 1974; King 1976) and it was suggested that in the case of grass grub this resistance may be caused by either a non-preference or an antibiosis mechanism or by both (Sutherland and Hillier 1974). The existence of potent feeding deterrents in *Lotus* major root for larvae of both scarab species was subsequently reported (Sutherland *et al* 1975; Sutherland 1976) indicating a role for a non-preference mechanism, though Farrell and Sweney's early (1972) suggestion of an antibiosis factor remained untested. Grass grub mortality in trials with lotus major was greater than might have been expected from starvation alone (due to feeding deterrents) so we decided to investigate the possible existence of a toxin for the larvae in *Lotus* root. The major obstacle to such a study was the difficulty in persuading larvae to ingest known quantities of lotus major root. This problem has now been overcome and we report here on the results of preliminary experiments and the techniques which made them possible.

MATERIALS AND METHODS

Lotus major ('Grasslands 4705'), lotus corniculatus (*L. corniculatus*, Maitland) and lotus hybrid (*L. corniculatus* x *L. pedunculatus*, Grasslands 4712') were grown outdoors in flower pots. Intact 250 g root samples were washed in cold water and homogenized in distilled water. The homogenate was filtered and the filtrate concentrated under vacuum at 45°C to a final concentration of 10 g per ml (lotus major) or 25 g per ml (lotus corniculatus, lotus hybrid). These aqueous crude extracts were stored at -20°C.

Field-collected third instar grass grub and black beetle larvae were starved for 24 hours prior to use. Test insects were not selected by weight but only for vigour. They were individually force-fed with measured doses of the crude extracts. This was accomplished by carefully spreading the mandibles and gently inserting a blunted hypodermic syringe needle through the mouth and down the gut. The syringe was held stationary in a micro-applicator which delivered accurate repeatable doses and which was operated by a foot switch. Disposable hypodermic needles were blunted and smoothed on a fine oilstone; 30 gauge needles for grass grubs and 27 gauge for black beetle larvae.

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Plant Resistance to Pests

Various doses were administered with a maximum of 20 μ l for grass grubs and 50 μ l for black beetle larvae. Control larvae were dosed in an identical fashion with distilled water. Larvae were kept separate and were inspected 24 hours after treatment, when they were classified as dead (no observable bodily movement upon stimulation) or alive. Mortality due to handling ranged from 2-5% and was reduced as we became more skilled at the dosing technique. Larvae which died from needle damage turned black and were thus easily distinguished from those which died from toxins. They were not included in the results.

RESULTS

Lotus major root contains a potent toxin for grass grubs and black beetle larvae. A single dose of crude root extract equivalent to 0.2 g root killed 100% of treated grass grubs in 24 hours (Table 1). The ED₅₀ of the extract was equivalent to 0.063 g fresh root. Lotus corniculatus root extract was considerably less toxic to grass grubs: it caused appreciable mortality only at high concentrations and had an ED₅₀ of 0.36 g root. The extract of lotus hybrid root was not toxic to grass grubs.

TABLE 1: TOXICITY OF THREE LOTUS SPECIES TO 3rd INSTAR GRASS GRUB LARVAE. FIFTEEN LARVAE WERE TESTED WITH EACH DOSE (EXPRESSED AS g FRESH ROOT)

	% mortality/dose					
	0.01 g	0.05 g	0.10 g	0.15 g	0.20 g	0.25 g 0.50 g
lotus major	0	36	77	92	100	100 —
lotus corniculatus	0	0	0	0	15	7 83
lotus hybrid	0	0	0	0	0	0 0

The effect of the crude extracts on black beetle larvae paralleled those on grass grubs (Table 2). Again lotus major was very toxic (ED₅₀ = 0.307 g); lotus corniculatus was about five times less active (ED₅₀ = 1.41 g) and the extract of lotus hybrid was not toxic under these conditions.

TABLE 2: TOXICITY OF THREE LOTUS SPECIES TO 3rd INSTAR BLACK BEETLE LARVAE. TWENTY LARVAE WERE TESTED WITH EACH DOSE (EXPRESSED AS g FRESH ROOT)

	% mortality/dose				
	0.025 g	0.25 g	0.50 g	1.00 g	2.50 g
lotus major	0	37	79	100	100
lotus corniculatus	0	0	5	30	95
lotus hybrid	0	0	0	0	5

A similarly prepared aqueous extract of lotus major foliage was even more toxic to both scarab species than that of the root. The foliage extract had an ED₅₀ of 0.02 g for grass grubs and 0.18 g for black beetle. At even the lowest concentrations larvae were noticeably affected, if not killed, by the extract.

There was no mortality in any test amongst control larvae dosed with equivalent volumes of distilled water.

DISCUSSION

This is the first report of a naturally-occurring toxin in a pasture plant for pasture pests. The compound from lotus major root is clearly very active because in our bioassay we only recorded mortality and did so 24 hours after a single dose, yet still found a low ED₅₀ for each insect. Whether or not lotus corniculatus root and lotus major foliage contain the same material but at different concentrations or whether they contain other toxic compounds remains unknown.

The implications of these results for plant resistance studies are clear. Previously we have isolated feeding deterrents which no doubt limit grass grub and black beetle ingestion of lotus major root in the field. Now there is unequivocal evidence of a toxin in that root, and the chemical mechanisms upon which the resistance is based become more certain. Furthermore, our results with the three *Lotus* species are in line with those of Farrell and Sweeney (1974) who measured grass grub growth in pot trials. They found that lotus major was resistant, lotus corniculatus less so, and lotus hybrid least of all. It is tempting, therefore, to conclude that root toxicity is indeed causally linked to resistance.

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