A CHEMICAL BASIS FOR PLANT RESISTANCE TO GRASS GRUB AND BLACK BEETLE LARVAE (COLEOPTERA : SCARABAEIDAE)

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Abstract

Crude extracts of root of the resistant pasture plants lucerne *(Medicago sativa)* and *Lotus pedunculatus* contain strong feeding deterrents for third instar *Costelytra zealandica* and *Heteronychus arator* larvae. Purified saponins isolated from active crude lucerne root extract markedly reduce grass grub feeding and have an ED_{50} of 0.019%. Black beetle larvae are even more sensitive to the material. Separate and chemically distinct feeding deterrent fractions, active against the two insects, have been isolated from Lotus *pedunculatus* root. The role of feeding deterrents in mediating the non-preference of these insect pests for lucerne and *Lotus* is discussed.

INTRODUCTION

THE FACT that some pasture plants such as lucerne ***(Medicago sativa)*** and Lotus *pedunculatus* suffer relatively little damage from grass grub and black beetle attack is well known to farmers and has been established experimentally (Farrell and Sweney, 1972, 1974a, b) If this resistance is to be fully exploited, however, the basic mechanisms involved must be discovered. The exploitation of plant resistance is of particular importance at present when other methods of control are unacceptable because of damage to the environment, or are unselective, inefficient, and/or uneconomical. But this objective will be fully realized only when plant breeders are in a positioa to breed varieties with increased levels of resistance to insects and this requires accurate information on the factors which are responsible for that resistance.

These factors fall into three possible categories: mechanical factors such as toughness of plant tissue or hairiness may limit feeding; chemical factors such as repellents or feeding deterrents may reduce feeding by making the plant distasteful; or toxins present in the plant may poison the insect. We are currently investigating the influence of feeding deterrents in Lotus *pedunculatus* ("Lotus" hereafter) and lucerne on the feeding behaviour of grass grub (*Costelytra zealandica*) and black beetle (*Heteronychus arator*) .

MATERIALS AND METHODS

Third instar C. ***zealandica*** larvae are stimulated to feed vigorously by the combined influence of sucrose and ascorbic acid (at concentrations of 0.1 M and 0.01 M, respectively) (Sutherland, 1971; Sutherland and Hillier, 1974) . Black beetle larvae are similarly stimulated by maltose (0.1 M) (Sutherland, 1976). A bioassay was devised in which larvae of both species were individually presented with agar/cellulose discs containing these stimulants either aloae (standard control) or together with various plant root extracts and fractions thereof. After 24 h the number of faecal pellets produced by each larva was counted to provide an accurate index of feeding (see Sutherland, 1971; Sutherland ***et** al.*, 1975a for details). The presence of feeding deterrents in the plant extracts was indicated by a significant reduction in faecel pellet output. Other larvae were given blank discs containing no stimulants or test extracts to provide a lower base-line of larval response.

RESULTS

GRASS GRUB

Crude extracts of lucerne and ***Lotus*** root had a marked effect upon the feeding response of *C. zealandica* larvae to the phagostimulants sucrose and ascorbic acid (Table 1). In both cases grubs which would otherwise have fed vigorously failed to do so. Their faecal pellet count was not significantly different from that of larvae in the blank control.

TABLE 1

Faecal pellets produced by 20 3rd instar C. *zealandica* and ***H. arator*** larvae in 24 h feeding on agar/cellulose discs. "Standard" = either 0.1 M sucrose + 0.01 M ascorbic acid (C. *zealandica*) or 0.1 M maltose (*H.* ***arator***).

	Blank	*Standard*	*Standard + Lotus*	*Standard + Lucerne*
C. zealandica	64	305	85	37
H. arator	132	740	183	137

The active principle in ***Lotus*** root has 'been followed through consecutive fractionations of the original crude extract (see Fig. 1) and at least two active fractions (50-A and 50-C) have been isolated. These are currently being further isolated and identified (Sutherland et al., 1975b).

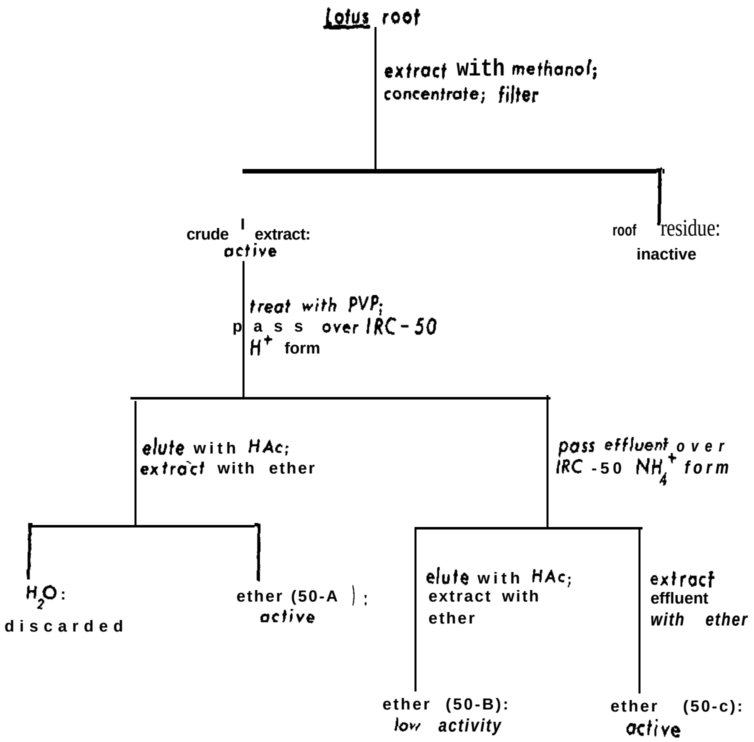

FIG. *1: **A scheme for the** isolation of two strong feeding* ***deterrents*** *for* Costelytra zealandica *larvae from* Lotus pedunculatus ***'Grasslands 4703'.***

Pure saponin isolated from crude lucerne root extract has been tested and is a 'highly active feeding deterrent for grass grub. It reduced larval feeding by 50% at a concentration of 0.019% (Fig. 2). Commercial saponin was 10 times less active, although still an #effective feeding deterrent. A second deterrent was isolated from lucerne root by extraction with chloroform. This component of the root has yet to be identified.

BLACK BEETLE

The strong response of black beetle larvae to 0.1 M maltose was also effectively nullified by the addition of crude extracts of Lotus and lucerne (Table 1) . Subsequent fractioaetion of the former has led to the isolation of three chemically distinct active fractions. In the case of lucerne the feeding deterrent is water-soluble and may again prove to be saponins. Certainly, black

TABLE 2

Faecal pellets produced by 20 3rd instar *H. arator* larvae feeding for 24 h on agar/cellulose discs containing 0.1 M maltose together wtih various concentrations of pure 'Wairau' lucerne saponins.

Blank	Standard	Standard + % of Added Saponins						
		0.001	0.005	0.01	0.05	0.1	0.5	1.0
179	729	386	369	410	308	343	244	149

beetle larvae are extraordinarily sensitive to pure 'Wairau' lucerne saponins (Table 2). At all concentrations tested there was a significant reduction in feeding and even at the lowest concentration, 0.001% (10 ppm) , feeding was reduced by approximately 50%.

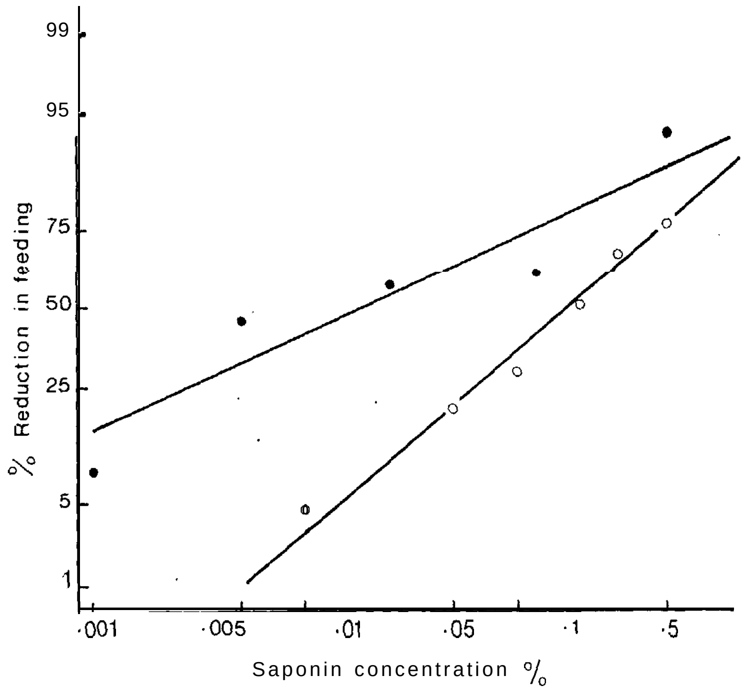

FIG. 2: Dose/response curves for lucerne var. 'Wairau' saponins (●) and commercial snponins (0) fed to 3rd instar *Costelytra zealandica* larvae.

DISCUSSION

The development of varieties of pasture plants which are highly resistant to grass grub and black beetle attack is an attainable objective. Already resistance among some grasses and legumes to grass grub and among legumes to black beetle has bsen demonstrated (Farrell and Sweney, 1972, 1974a, b; Wallace, 1946). But an essential basis for a plant breeding programme to achieve this objective is accurate informatioa cn the factors conferring that resistance. We have demonstrated the presence ad potent feeding deterrents in the root of resistant *Lotus pedunculatus* and lucerne. Because these markedly reduce feeding rates we have no doubt that they play an important role in controlling resistance in the field. In lucerne, a preliminary study of the weight gain of grass grubs feeding on 13 cultivars provided some evidence linking poor growth with high root saponin levels (Farrell and Sutherland, unpubl. data) but the results were not conclusive and there seems little doubt that more than this one factor is implicated. Nevertheless, saponin levels can be easily modified by selective plant breeding (Pedersen et al., 1973) and may be the key to the development of highly resistant varieties of lucerne. Saponins of diverse composition are also found in a number of plant species besides those in the Leguminosae and a study of the effects of these on insect biology could reveal novel deterrent compounds.

Similarly, once the feeding deterrents in Lotus have been identified, a wider range of potential resistance factors will be available for plant breeders to utilize. Whether these deterrents also occur, perhaps at lower levels, in other plants remains to be seen. If they do, the prospects for the development of new resistant varieties seem good. Furthermore, since the transfer of genetic material from one species to another has now been demonstrated (Pandey, 1975) the incorporation of resistance-promoting factors into previously susceptible species becomes conceivable.

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