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BIOCHEMICAL MECHANISMS OF PEST RESISTANCE IN PASTURE LEGUMES

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ABSTRACT

The resistance of legumes to grass grub larvae (*Costelytra zealandica*) has been studied under controlled environment conditions and the resistance mechanisms have been investigated by chemical fractionation of root extracts using quantitative bioassays. The feeding deterrent activity of *Lotus pedunculatus* arises from the presence of both nitroesters and non-polar fractions including the isoflavan and vestitol. Bean (*Phaseolus vulgaris*) has been shown to be resistant to grass grub and this has been related to the presence of the isoflavonoid feeding deterrent, phaseollin, in the roots. An understanding of chemical defence mechanisms could lead to the development of new methods in selection or genetic manipulations to develop resistant white clover (*Trifolium repens*).

KEYWORDS

Grass grub, *Costelytra zealandica, Trifolium repens, Lotus pedunculatus, Phaseolus vulgaris,* plant resistance, resistance mechanisms, nitroesters, isoflavonoids, phaseollin.

INTRODUCTION

One of the constraints on maximum pasture production in New Zealand is the effect of the insect pest, grass grub (*Costelytra zealandica*), whose subterranean root feeding larvae cause significant damage to white clover (*Trifolium repens*). As an important component of an integrated control strategy, the development of a white clover which is either resistant to the insect and adversely affects larval growth, or tolerant to the insect and grows well despite larval attack, remains a significant goal for pastoral research in New Zealand. So far, the intensive search to identify sources of resistance from within white clover ecotypes or cultivars has had inconclusive results (Wilson 1978a, b; Wilson and Farrell, 1979; Van den Bosch and Gaynor, 1986). This means that classical selection and breeding programmes have not yet made major progress toward the development of grass grub-resistant wh. clover.

Apart from white clover, several other crop legumes have been identified as resistant to grass grub (Farrell and Sweney 1972, 1974). We have investigated the chemical basis and mechanism of resistance of some of these plants. The long term aims of this research have been to understand the biological phenomena involved and to provide a chemical basis for any future selection or hybridisation programmes. An understanding of the chemistry, biochemistry, and molecular genetics of resistance will be required for specific gene transfer using recombinant DNA technology to produce resistant clovers combining the preferred genetic attributes of several species.

Our initial studies on resistant plants resulted in the isolation and identification of grass grub toxins and feeding deterrents which could contribute to the classic modalities of genetic resistance recognised by Painter (1951), namely antibiosis and non-preference. Nitropropanoyl glucose esters and saponins were isolated as toxins from Maku lotus (*Lotus pedunculatus*) and lucerne (*Medicago sativa*) respectively (Hutchins *et al.,* 1984; Sutherland *et al.,* 1975a, 1982), and isoflavonoids were isolated as feeding deterrents from Maku lotus (Russell *et al.,* 1978), white lupin (*Lupinus angustifolius*) (Lane *et al.,* 1985a) and sainfoin (*Onobrychis viciifolia*) (Russell *et al.,* 1984). A detailed structure-activity study (Lane *et al.,* 1985b) on a range of isoflavonoids from various plants indicated that feeding deterrent activity is critically dependent on overall molecular shape and the arrangement of particular substituent groups. The most active compounds were the complex isoflavonoids such as phaseollin, found in French bean (*Phaseolus vulgaris*).

Having identified some toxins and/or feeding deterrents from resistant legumes, we set out to evaluate their significance in resistance and we report here the results of our studies with Maku lotus and French bean.

RESULTS AND DISCUSSION

Measurement of lotus and bean resistance

The resistance of Maku lotus in terms of grass grub survival and larval weight gain with field and pot grown material had been well established (Farrell and Sweney, 1974). However, the resistance of beans had not been determined. On the basis of the isoflavonoid content of the roots (Sutherland *et al.,* 1980) we predicted that French bean would be resistant to grass grub attack. The complex isoflavonoid, phaseollin, which is extremely active in the feeding deterrent assay ($FD_{50}=0.05$ ppm; Lane *et al.,* 1985b), is found in the roots at levels (3-5 ppm) at which larval feeding and hence growth were expected to be reduced to a low level.

To test whether beans were resistant, the growth of third instar grass grub larvae on dwarf beans (Tendergreen) was compared with that on lotus (Grasslands Maku) and white clover (Grasslands Huia). The latter were included as resistant and non-resistant controls, respectively. Third instar larvae feed actively between April and July, the period during which field-grown beans senesce. To overcome this seasonal difference between bean and grass grub growth patterns, and to provide conditions favourable to the growth of both plants and insects, the experiment was conducted in a controlled environment (18°C and 14 hour day length). Ninety two plants of each species were established singly in pots and one larva of known weight was buried beneath the surface of each pot as described by Gaynor *et al.* (1985).

After 28 days the larvae were removed from the pots and reweighed. Overall recovery was 95%. The larval weight gains were significantly different between all three plant species (Fig. 1); the weight gain on beans (78 mg) was lower than on white clover (108 mg), but not as low as on lotus (62 mg). This indicates, as predicted on the basis of the root chemistry, that beans are resistant to grass grub by comparison with white clover.

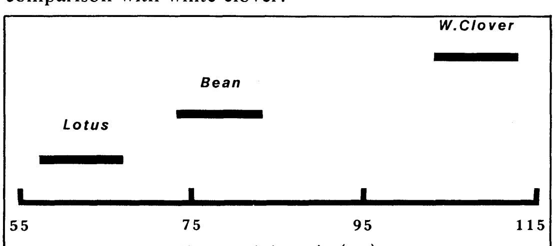

Figure 1. Growth of third instar *Costelytra zealandica* larvae under three legumes for 28 days. Bars show 95% confidence intervals.

Chemical basis of bean resistance

Extracts of the bean, lotus, and white clover roots from this experiment were tested for toxicity (Table 1) following the procedures of Hutchins *et al.* (1984). As observed previously (Sutherland *et al.,* 1982) the lotus extract was toxic and the white clover extract was not.

Table 1. Percent mortality among 20 third instar *Costelytra zealandica* larvae dosed with crude extracts of roots from three legumes (25 g fresh wt/2.5 ml) and a water control (from Gaynor *et al.,* (1985)).

Plant species	% Mortality
Clover	10
Bean	25[1]
Lotus	85
Control	10

[1] 15% of these larvae (3) died due to damage by the hypodermic needle.

There was no evidence of any significant toxic effect of the bean extract, hence bean resistance does not appear to be due to the presence of toxins.

The bean root extract showed feeding deterrent activity (Table 2) in the faecal pellet bioassay of Sutherland and Hillier (1974), as did extracts from the other two species. The activity of lotus root extracts in this assay is well documented (Sutherland *et al.,* 1975b; Russell *et al.,* 1978). The white clover extract was also active but at concentrations two-fold higher than for lotus. Because of the high variability of the data for the bean extract, quantitative comparison with the other species was not possible.

Table 2. Concentration of crude root extract (g fresh wt/g medium) which reduced feeding of third instar *Costelytra zealandica* larvae by 50% (FD_{50}), (from Gaynor *et al.,* (1985)).

Plant species	FD_{50} (c)[1]
Clover	0.37 (1.56)
	0.59 (1.23)
Bean	0.37 (2.67)
	0.10 (2.4)
Lotus	0.16 (1.39)
	0.26 (1.2)

[1] The error limits are FD_{50} x/ ÷ c

To further investigate feeding deterrent activity of bean roots, a chemical fractionation was undertaken. Bean roots were extracted with enthanol, the extract was fractionated, and the activity of the fractions measured (Fig. 2). Most of the high feeding deterrent activity found was attributable to phaseollin. The closely related isoflavonoids phaseollinisoflavan and 2'-O-methyl-phaseollinisoflavan accounted for much of the remaining activity.

Thus the resistance of bean to grass grub appears to be due to the presence in the root of the highly active isoflavonoid feeding deterrent, phaseollin, and related compounds, as we suggested.

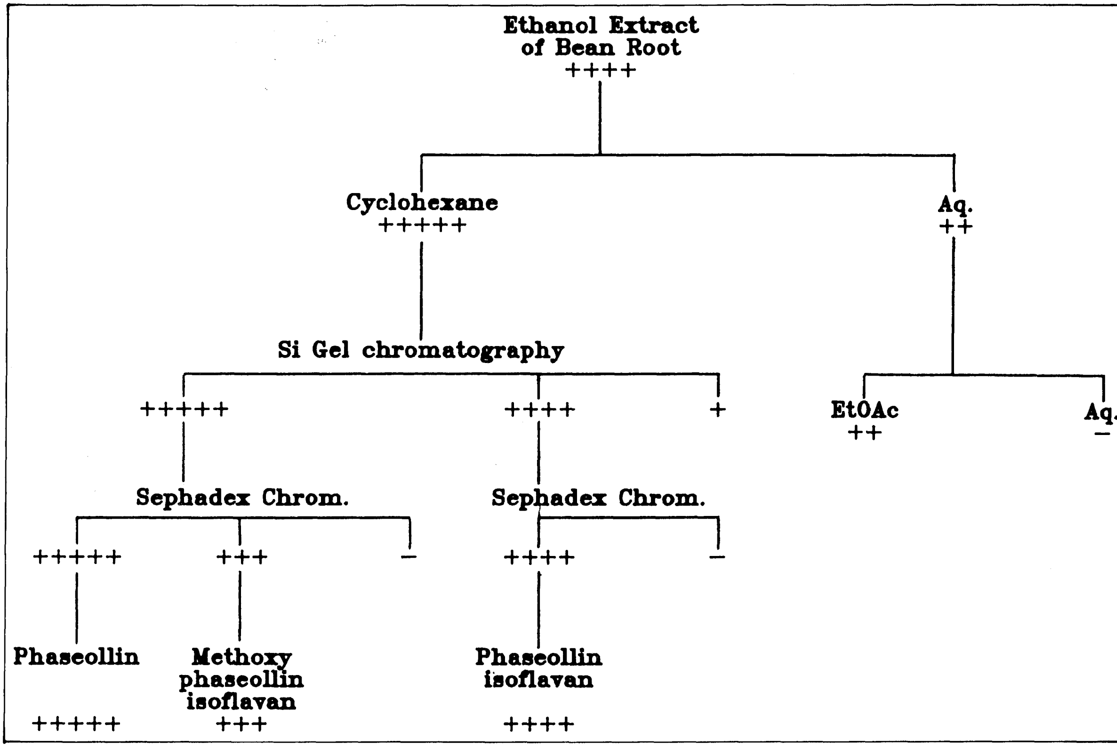

Figure 2. Fractionation of bean (*Phaseolus vulgaris*) root extract. Feeding deterrent activity expressed as relative activity defined as 100 x ratio of FD_{50} (root extract) to FD_{50} (fraction); + + + + + >120; + + + + 120-60; + + + 60-30; + + 30-15; + 15-7; − <7.5.

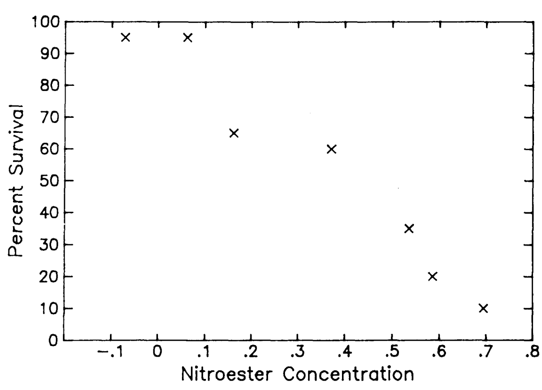

Figure 3. Grass grub survival after dosing with root extracts (10 μl, 10 g/g fresh weight) from several lotus samples. Nitroester concentration is expressed as log 3-nitropropionic acid, mg/g fresh weight of tissue.

Chemical basis of lotus resistance

Previous work (Sutherland *et al.*, 1982) has shown that the crude extract of lotus root is both toxic and a feeding deterrent to grass grubs. Hutchins *et al.* (1984) have shown that the acute toxicity of lotus roots can be attributed to the glucose nitropropanoyl esters of which karakin (glucose trinitropropanoyl ester) is the principal component (80% of ester content). When the root extracts of several *Lotus pedunculatus* root samples containing different levels of nitroester were tested for toxicity by injection (10 μl, 10g fresh wt/ml) into the foregut of grass grub, a correlation was observed (Fig. 3) between toxicity (survival after 24 hours) and nitroester concentration (determined as nitrite, Greenwood, 1984). The nitroester content of the root extracts does account for the toxicity observed (Lane, unpub. data), indicating the nitroesters are indeed the main toxins in the plant as Hutchins *et al.* (1984) suggested.

Karakin, however, also shows feeding deterrent activity in the faecal pellet bioassay (Sutherland *et al.*, 1982). The correlation between feeding on the root extracts of a number of plants and their nitroester content is shown in Fig. 4. While there is considerable variation in this behavioural bioassay, the reduction in feeding is correlated with the nitroester content of the extract. The nitroester

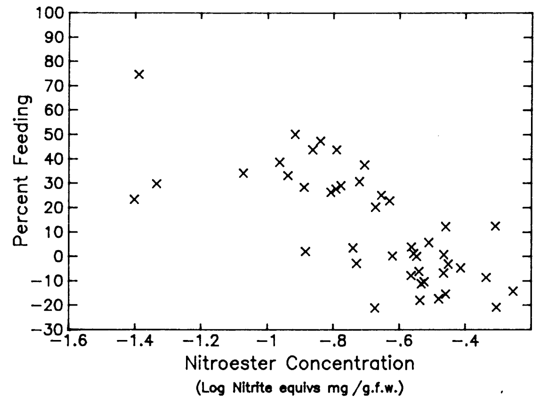

Figure 4. Grass grub feeding activity on root extracts from several lotus samples (1 g fresh wt/ml medium). Nitroester concentration is expressed as log 3-nitropropionic acid, mg/g fresh weight of tissue.

concentrations at which feeding is completely inhibited are considerably lower than the concentrations at which toxic effects are first observed (Sutherland *et al.,* 1982). This fact, together with the previous observation that in 28-day pot trials of Maku lotus, under controlled conditions, grass grub survival is high (95%), suggests that feeding deterrent activity is more important than acute toxicity in resistance and that non-preference may be the principal mechanism by which the nitroesters exert their effect. The possibility of chronic toxicity affecting the overall survival and fitness of the insect cannot be excluded but this parameter has not been measured at this stage.

Extracts of lotus roots containing low nitroester concentrations remain relatively unpalatable (Fig. 4), which suggests that other deterrents are present in Maku lotus. In fact the isoflavin, vestitol, has been isolated from the pots as a major feeding deterrent (Russell *et al.,* 1978). To determine relative contributions to feeding deterrent activity, a crude lotus root extract was fractionated and the activity of each fraction measured (Fig. 5). The feeding deterrent activity appears to be due equally to the nitroesters, predominantly karakin, and to the isoflavan, vestitol, together with other materials of similar polarity.

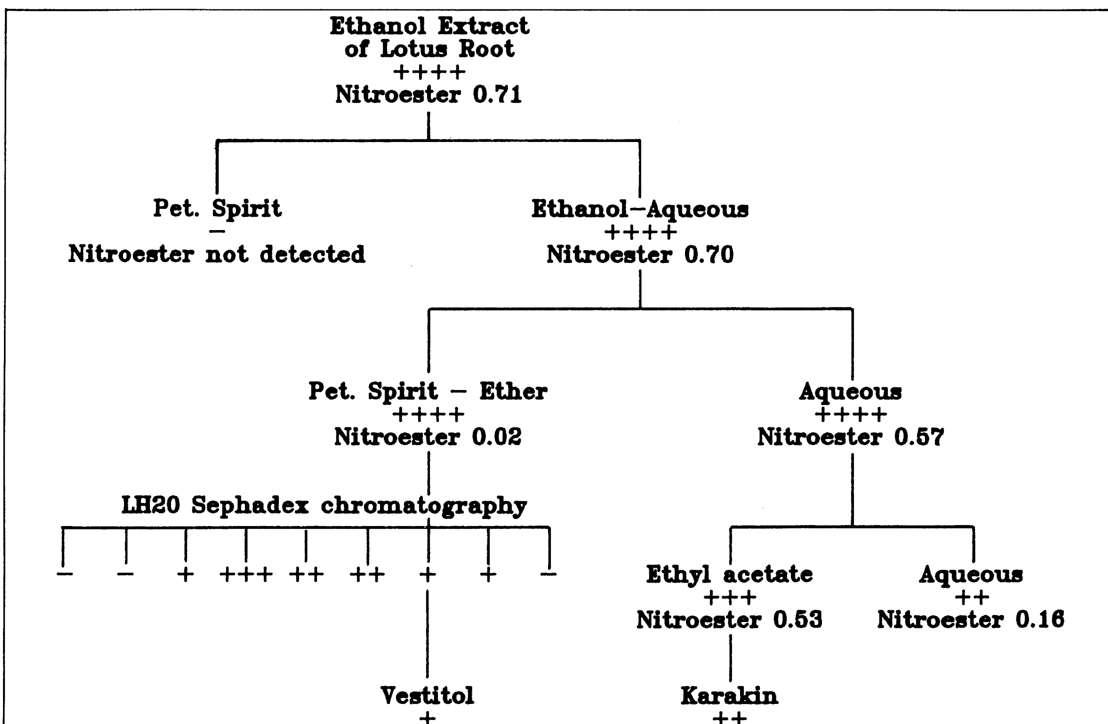

Figure 5. Fractionation of *Lotus pedunculatus* root extract. Nitroester content expressed as mg nitrite/g fresh wt determined colorimetrically (Greenwood, 1984). Feeding deterrent activity expressed as relative activity defined as 100 x ratio of FD_{50} (root extract) to FD_{50} (fraction); + + + + >60; + + + 60-30; + + 30-15; + 15-7.5; − <7.5.

Karakin, which is of comparatively low feeding deterrent activity (FD_{50} 0.1%), affects the feeding behaviour of grass grubs toward lotus roots because of its high yield (0.02% fresh wt), while vestitol, in spite of its low yield (3 ppm), has a significant effect because of its high feeding deterrent activity (FD_{50} 10 ppm).

Feeny, (1976), introduced the concept of quantitative and qualitative chemical defences in plants when he discussed the effects on insects of 'digestibility reducing' components, such as tannins, present in high yields as opposed to highly active toxins, present in low yields. The nitroesters in lotus roots can be considered quantitative resistance factors affecting grass grub feeding because of their relatively high yield. The isoflavonoids can be considered qualitative resistance factors affecting the grass grub feeding because of their high activity, in spite of their low yield. Quantitative chemical defences should provide persistent resistance to a range of insects but at considerable metabolic cost to the plant because of the high yields required. On the other hand, a qualitative defence could retain the desirable agronomic production characteristics yet introduce effective resistance. In either case the possibility of deleterious side effects on other herbivores needs to be considered. In clover, isoflavonoid pathways already exist and qualitative resistance could be introduced by genetically modifying these pathways to produce highly active feeding deterrents such as vestitol or phaseollin.

CONCLUSIONS

We have shown that beans are resistant to grass grub and have related this to the presence of the highly active feeding deterrent, phaseollin, in the roots. Further, we have shown that the resistance of Maku lotus is multifactorial involving both isoflavonoid and nitroester feeding deterrents. These studies have enabled us to recognise non-preference and antibiosis resistance mechanisms, with respect to grass grub, and to suggest that quantitative and qualitative chemical defence mechanisms are found in lotus and bean. We are encouraged to proceed with research on the biosynthesis of complex isoflavonoids such as phaseollin with a view to modifying isoflavonoid production in white clover. Our work towards the development of a grass grub-resistant clover is thus proceeding through a study of the biochemical mechanisms which need to be delineated before any molecular genetic techniques can be applied to plant breeding programmes.

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