INSECT ANTIFEEDANTS - A NEW ZEALAND PERSPECTIVE

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SUMMARY

New Zealand has a unique native flora that exhibits a high incidence of monophagy. Eighteen plants with low insect associations have been screened for antifeeding activity against greenheaded and brownheaded leafroller larvae (*Planotortrix octo* and *Ctenopseustis obliquana*), tropical armyworm (*Spodoptera litura*) and ant (*Technomyrmex albipes*). Antifeeding activity was assessed using a two-way choice bioassay. Nine plant species showed significant activity and these were chemically examined by activity-guided isolation or from assessing the activity of known bioactive constituents.

Keywords: insect antifeedants, greenheaded leafroller, brownheaded leafroller, tropical armyworm, ant, plant extracts, phytochemicals.

INTRODUCTION

There is increasing scientific interest in the roles plant secondary metabolites play as cues of insect behaviour, particularly in relation to host recognition and feeding. While plant metabolites may produce toxic effects if ingested, antifeeding activity leading to rejection of the plant as a suitable host may be of greater significance in determining the extent of insect herbivory.

The utilization of antifeedants to disrupt feeding behaviour is only at a preliminary stage, but interest in this area is growing as part of the search for more ecologically acceptable pest control strategies. Antifeedants are of particular interest due to their potential for high species specificity, for integration with pest management schemes, and for disrupting host-plant recognition through exploiting recent developments in the genetic modification of plant biosynthetic pathways.

The New Zealand flora is a unique example of plants that have evolved only with insect and avian herbivores. In the 80 million years since New Zealand has been isolated, a high incidence of host specificity has developed and over 60% of New Zealand phytophagous lepidoptera are restricted to plants of one genus or species (Dugdale 1975). That is, the majority of plants are unsuitable hosts to most insects except the species that is adapted to it. This is a higher proportion than any other flora in the world and emphasises the long association and co-adaptation that has occurred. The New Zealand flora is likely to be a rich source of insect active chemicals, and in earlier searches, insect moulting hormones and other compounds which disrupt insect growth and development were isolated (Russell and Fenemore 1970; Singh *et al.* 1978). More recently, 21 native plants were screened for repellent activity to the wool pest *Tinea dubiella* (Gerard and Ruf 1991) and two, ngaio (*Myoporum laetum*) and porokaiwhiria (*Hedycarya arborea*), strongly inhibited oviposition.

In previous work, we have identified highly active antifeedants for the endemic pest, grass grub (*Costelytra zealandica* White), from resistant pasture legumes (Sutherland *et al.* 1981). Structurally related grass grub antifeedants have been isolated from the native legumes, kowhai (*Sophora microphylla*) and native broom (*Carmichaelia* spp) and are now reported below. We have extended the search for novel antifeedants by seeking plants within the New Zealand flora which are deterrent or repellent to the greenheaded and brownheaded leafrollers (GHLR and BHLR, *Planotortrix octo* Walk. and *Ctenopseustis obliquana* Walk.), endemic pests of horticultural crops. We have selected a group of New Zealand plants recognised by

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ecologists to have few insects associated with them. Many of these plants are the endemic remnants of tropical genera which have survived and evolved under temperate conditions. Alcoholic extracts of these plants have been assayed for insect antifeeding activity to the two leafrollers and in some cases to the tropical armyworm (TAW, *Spodoptera litura* Fabr.) and the ant (*Technomyrmex albipes*) in supplementary assays. Some of the active plants which have not been hitherto examined chemically have been investigated using a bioassay-directed isolation.

MATERIAL AND METHODS

The two-way choice test design of Blaney *et al.* (1990) was used for GHLR, BHLR and TAW, whereby fifth instar larvae were presented with diet squares or disks incorporating an extract of the plant material. Antifeeding activity was assessed by recording the weight of test square eaten after 24 h compared to a control square. An antifeedant index ($(C-T)/(C+T)$) was calculated from the mass eaten of control (C) and test (T) disks/squares, respectively. A potent antifeedant would have a positive index greater than 0.5. A negative index indicates a stimulant. The data was subjected to an analysis of variance using a randomised block design.

The diet squares for GHLR and BHLR were prepared by spreading hot standard rearing diet (Singh, 1983) on 20 x 20 cm glass plates with a 'thin-layer' spreader set at 1 mm thickness, cutting the cooled diet into 15 mm squares and freeze drying. The squares were moistened with 70 μ l of water before presentation to the insects and dried in a desiccator after the feeding test. For TAW, 2 cm glass fibre filter disks were made palatable by adding an aqueous solution (0.1 ml) of lima bean extract containing 50 mM sucrose. The test disk contained in addition, 0.1 ml of the plant extract.

The ant choice test used drops (50 μ l) of 40% sucrose solution incorporating the plant extracts with a sucrose only, control (Ellis pers. comm.). Numbers of ants feeding at a particular drop after 5, 10 and 15 minutes enabled an assessment of antifeeding or repellent activity (Feeding index = ants feeding at the test drop/total number of ants feeding).

For grass grub, the faecal pellet bioassay (Sutherland and Hillier 1974) was used to assess antifeeding activity. The 24 h faecal pellet counts of third instar larvae feeding on cellulose/agar/sucrose disks containing plant material were compared with those from control disks containing sucrose only.

Plant material was obtained from various sites and its identity authenticated. The plants were either freeze dried or air dried in a forced draft oven, and ground to a fine powder. The plant extracts used in the antifeeding assays were prepared by Soxhlet extraction with methanol and evaporation to dryness. These were incorporated at the rate of 0.5% for GHLR, BHLR and TAW, and at 0.1% for ant and grass grub.

Where sufficient amounts of pure compounds were obtained from the separations or were available from previous chemical examinations of the same plant, these were tested in the appropriate assays at a range of concentrations. This enabled a dose response curve to be calculated for a particular compound and gave a preliminary idea of the potency of the components. A more detailed examination of the efficacy of the isolated antifeedants is currently being pursued, concurrently with the isolation of further active compounds.

RESULTS AND DISCUSSION

Antifeedant bioassays usually depend on the ability to measure the amount of material consumed or not consumed by the insect. Since insects respond differently to food plants, antifeeding compounds for one insect may be active with another. Not only is the design of test important for a particular insect but it is also important to test a plant with a number of different insect species to identify a range of behaviourally-active metabolites.

The results from offering diet squares or disks containing extracts of eighteen native New Zealand plant extracts to five insect species, are given in Table 1. Of the tests carried out to date, fourteen plants showed activity against at least one insect, and

five plants showed antifeeding activity toward at least three insect species. These were *Macropiper excelsum* (kawakawa), *Sophora microphylla* (kowhai, root bark), *Coryocarpus laevigatus* (karaka), *Dysoxylum spectabile* (kohekohe) and *Podocarpus nivalis* (snow totara). This result may indicate the presence of antifeeding compounds in these plants that affect a broad spectrum of insects. A summary of our findings on the active plants together with an outline of the current knowledge of their insect-active metabolites follows:

***Sophora microphylla* (kowhai)**

While the foliage of kowhai did not show activity toward the test insects, the root bark was highly distasteful to GHLR, BHLR and grass grub larvae. Kowhai is host to several phytophagous lepidoptera but it was identified as a source of insect antifeedants in studies with grass grub. A bioactivity-guided separation with grass grub gave a series of complex isoflavanones and pterocarpanes. Isosophoranone and a novel 3-hydroxy isoflavanone deterred grass grub feeding at 200 ppm, while maackaia gave an FD_{50} of 50 ppm (concentration required to reduce feeding by 50% as measured from a dose response curve) (Lane *et al.* 1985). These results are consistent with structure activity studies on isoflavanoids which indicated that the pterocarpanes and isoflavones with a ring B prenyl moiety had high antifeeding activity toward grass grub larvae (Lane *et al.* 1985). There may be other classes of antifeedants present as GHLR was insensitive to maackaia and isosophoranone at 500 ppm in the choice bioassay.

***Carmichaelia* spp. (native broom)**

In the course of our studies with grass grub antifeedants from legumes, we found the roots of the native brooms contained active antifeedants. The leaf extract is not active toward GHLR and BHLR but we have yet to test root extracts with these insects. The most active grass grub antifeedants isolated from the roots were the isoflavanoids, vestitol (FD_{50} : 10ppm), isomucronulatol (FD_{50} : 10ppm) and medicarpin (FD_{50} : 40ppm).

***Pseudowintera colorata* (horopito)**

The dull green, red blotched leaves of this shrub are very pungent to the taste but the extract although repellent in the ant assay, failed to give a significant antifeeding response to GHLR and BHLR at the concentration tested. Gerard *et al.* (1992) have investigated this plant for its insecticidal properties against the webbing clothes moth (*Tineola bisselliella*) and the Australian carpet beetle (*Anthrenocerus australis*). The active components were identified as the terpenoids polygodial and 9-deoxymuzigadial and both showed significant antifeeding activity at concentrations on wool of 400 ppm. Polygodial is a well known insect antifeedant against a number of lepidopteran species (Kubo *et al.* 1976) and it has been suggested that the activity is caused by a Michael type addition to a nucleophilic terminal of a taste receptor since loss or epimerisation of the dialdehyde groups result in a loss of activity (Kubo and Nakanishi 1977). Its activity toward leafroller larvae and TAW has yet to be evaluated although it is known to have strong antifeedant activity against other *Spodoptera* species (Kubo *et al.* 1976).

***Litsea calicaris* (mangeao)**

The root bark of this plant was deterrent toward TAW and ants but the leafrollers did not give a significant response. A bioassay-directed isolation indicated that the alkaloid fraction contained the activity. Aporphine alkaloids (boldine, norboldine, and actinodaphine), typical of the Lauraceae, were the main active components. Norboldine gave a positive feeding index of 0.6 at 500 ppm with GHLR indicating strong antifeeding activity at this concentration. The aporphine alkaloid, isoboldine has previously been shown to be completely inhibitory to tropical armyworm at 200 ppm (Munakata *et al.* 1977) and we have found that a related alkaloid, dicentrine, from *Litsea excelsa* has a feeding index of 0.6 at 0.1% concentration, with TAW.

***Macropiper excelsum* (kawakawa)**

Extracts of kawakawa showed antifeeding activity with GHLR, BHLR and ants. Several insect-active constituents have previously been isolated from this plant (Russell and Fenimore 1973; Nishida *et al.* 1983). Myristicine, the main bioactive constituent of the leaf material, did not show antifeeding activity toward the GHLR

at 500 ppm. Other bioactive constituents yet to be tested include juvadenecene which has insect juvenile hormone activity (Nishida *et al.* 1983) and the series of methylenedioxy lignans (Russell and Fenemore 1973) which are related to the juvenile hormone mimic, sesomolin. A systematic antifeeding directed separation may uncover further bioactive constituents.

TABLE 1: Insect antifeeding activity of some New Zealand plants.

Plant	Plant part	GHLR	BHLR	TAW	ANT	GG
Winteraceae: <i>Pseudowintera colorata</i>	leaf	•	•		+	
Lauraceae: <i>Bielschmedia tawa</i>	leaf	•	-			
<i>Litsea calcaris</i>	root bark	•	•	+	+	
	leaf			-		
Piperaceae: <i>Macropiper excelsum</i>	leaf	+	+	•	+	
Proteaceae: <i>Knightia exelsa</i>	leaf	•	-			
Passifloraceae: <i>Tetrapathea tetrandra</i>	leaf	•	-		+	
Elaeocarpaceae: <i>Elaeocarpus dentatus</i>	leaf	•	-	-	+	
Escalloniaceae: <i>Carpodetus serratus</i>	leaf	•	•			
Papilionaceae: <i>Sophora microphylla</i>	leaf	-	•	-		
	root bark	+	+	-	-	+
<i>Carmichaelia</i> spp	leaf	-	•			
	root					+
Corynocarpaceae: <i>C. laevigatus</i>	leaf	-	+		+	
	fruit					+
Meliaceae: <i>Dysoxylum spectabile</i>	fruit	+	+	+	+	
	leaf	+	+			
Sapindaceae: <i>Alectryon excelsus</i>	leaf	+	+	-		
Apocynaceae: <i>Parsonsia heterophylla</i>	leaf	+	-			
Verbenaceae: <i>Vitex lucens</i>	leaf	-	-	-		
Podocarpaceae: <i>Podocarpus nivalis</i>	leaf	+	+		+	
<i>Lepidothamnus intermedius</i>	leaf	•	•		+	
<i>Phyllocladus trichomanoides</i>	leaf	-	•		+	

* Based on the observed karakin content.

GHLR (Greenheaded Leafroller), BHLR (Brownheaded Leafroller), TAW (Tropical Armyworm). Choice test, feeding index (C-T/C+T): += +1 to +0.25 (deterrent), • = +0.25 to -0.25 (no significant effect), - = -0.25 to -1 (stimulent). ANT. Choice test, feeding index (no. feeding test/ total no. feeding): += 0 to 0.25 (deterrent), - = 0.25 to 1. GG (Grass Grub). Faecal pellet bioassay: + = significant reduction in pellet count compared to controls.

***Corynocarpus laevigatus* (karaka)**

The leaves and fruit of this plant were antifeedant toward the BHLR and ants. Prime candidate components, yet to be assayed with these insects are the 3-nitropropionyl esters of glucose, such as karakin and cibarian, which are responsible for the toxicity of the fruit to monogastric animals. Karaka produces eleven nitropropionates, five of which, have been isolated and identified (Moyer *et al.* 1979). These esters also occur in several legumes such as, indigofera, lotus, and the vetches, where their effects have been well studied (Byers *et al.* 1986). The esters are not only toxic to the grass grub but also antifeeding at 0.06%, a concentration 10-fold less than the LD₅₀ concentration (Sutherland *et al.* 1981). Ecologically, the antifeeding response is probably the more important.

***Dysoxylum spectabile* (kohekohe)**

The fruit of this plant was strongly deterrent to GHLR, BHLR, TAW and ants, and the leaves, tested against GHLR and BHLR, were also active. The dried powdered

plant material also dramatically affects the growth and development of leafroller when incorporated into diet (unpublished results). These results from the sole New Zealand member of Meliaceae are not unexpected as this plant family has yielded many insect-active terpenoid compounds (Champagne *et al.* 1992), including the well known antifeedant, azadirachtin. An extract of kohekohe fruit yielded a hexane partition fraction, rich in sesquiterpenes while reversed phase chromatography of the methanol/aqueous partition phase gave fractions containing limonoid terpenes (unpublished results). The limonoid and sesquiterpenoid fractions deterred both ants and TAW from feeding. A novel terpene, identified as 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol, was obtained from the latter fraction which repelled ants at 0.1% but it is inactive towards both leafroller species. Further work is proceeding on the other active constituents from this plant.

***Alectryon excelsus* (titoki)**

The leaf extract of titoki gave a low feeding index (*ca* 0.3) toward GHLR and a high feeding index (*ca* 0.6) toward BHLR. The essential oil of titoki is known to contain cyanolipids which release hydrogen cyanide (Eyres and Brooker 1976) and these are candidates to be examined for antifeedant activity. Cyanogenic glycosides are also found in the native passion vine (*Tetrapathea tetrandra*) (Russell and Reay 1972) but this plant was inactive toward the leafrollers. Its repellent activity toward the ants will need to be investigated.

***Parsonsia heterophylla* (kaihua)**

The leaves of kaihua showed antifeeding activity toward GHLR. Kaihua contains the unusual and toxic pyrrolizidine alkaloids, parsonsine and heterophylline (Edgar *et al.* 1980), and the crude alkaloid fraction was active towards GHLR at 0.1%. Further work is needed to establish these alkaloids as the antifeedant principles. Danine butterfly larvae, herbivores of tropical *Parsonsia* species, sequester pyrrolizidine alkaloids as precursors of adult semiochemicals (Edgar *et al.* 1980).

***Podocarpus nivalis* (snow totara)**

The extracts of the leaves of this plant showed antifeeding activity toward GHLR, BHLR and ants. This activity may be due to the norditerpene lactones isolated from snow totara leaves as dietary toxins to housefly and lightbrown apple moth larvae (Russell *et al.* 1973). The isolated compounds were toxic at low concentrations, nagilactone C gave a LD₅₀ of 12 ppm for housefly and 76 ppm for LBAM while hallactone A gave a LD₅₀ of 4 ppm for housefly (Singh *et al.* 1979). Although nagilactone C was not significantly deterrent to GHLR at 0.1% the dose response curve suggests that this compound is an antifeedant toward this insect.

***Lepidothamnus intermedius* (yellow silver pine)**

The foliage of this plant was deterrent to ants but did not show significant antifeedant activity toward GHLR and BHLR. The foliage of this plant contains up to 3% phytoecdysteroids (Russell *et al.* 1972) and these compounds would be expected to present a considerable biochemical problem to insects attempting to eat the plant, since ecdysones initiate the moulting cycle in insects. It would be anticipated that insects should learn to avoid ingesting large amounts of exogenous steroid that would interfere with their metabolic processes (Champagne and Bernays 1991). The principle ecdysteroid in yellow silver pine is 20-hydroxyecdysone and feeding tests with the housefly larvae showed that at 100 ppm, 20-hydroxyecdysone gave considerable pupal deformity and mortality (Singh *et al.* 1982) and that some antifeeding activity was apparent. With GHLR, 20-hydroxyecdysone at 0.1% gave a feeding index of 0.45 in the choice test, indicating moderate antifeedant activity at this concentration. The ant repellent test, on the other hand, showed that it was not the ecdysteroid fraction that was active but volatile methyl cinnamate and ethyl cinnamate. Methyl cinnamate repelled ants at a concentration of 0.1% in sugar solution.

Application of insect antifeedants

This insect antifeedant study in the New Zealand flora, although at an early stage, provides encouraging evidence of new sources of insect active compounds, new modes of activity for known insect-active compounds, and new active constituents

from non-host plants. Several active plant extracts needing closer investigation have been identified and a number of active alkaloids, terpenoids and phenolic compounds identified for further evaluation. The results suggest a wider search for highly active and specific antifeedants for leafroller is warranted.

The requirements for a useful antifeedant are dependant on the control strategy envisaged. Potentially, insect antifeedants can be used in pest control in two ways; as applied control agents and/or as factors for developing host-plant resistance. In the first case, new naturally occurring antifeedants are of value primarily as prototypical structures for new synthetic antifeedants. In the second case, the biosynthetic relationships between the antifeedant and the host-plant metabolites are of greater significance. There are as yet, few reports showing that antifeedants are being successfully used in commercial pest management strategies and the application of natural antifeedants is not yet a practical control option. However, there is much research to show that the limonoid antifeedant, azadirachtin, from neem (*Azadirachta indica*) is an effective control agent for lepidopteran pests (Smutterer and Ascher 1987) and commercial extracts of neem are available but still await registration in the USA. Pickett *et al.* (1991) have also demonstrated that polygodial can be used in the field to reduce barley yellow dwarf virus transmission by the bird-cherry aphid and the clerodane diterpene, ajugarin, can effectively control the mustard beetle in the field.

Antifeedants work by causing the pest insects to stop feeding and perhaps starve or to move from the treated crop to another plant. The development of stimulo-deterrent strategies incorporating several control technologies offer good prospects for the wider use of antifeedants. Application of an antifeedant to the developing crown of a crop plant causes insects to move to the lower leaves or to a trap crop planted in close proximity where a slow acting insecticide can be applied (Pickett *et al.* 1992). The limiting factor in the wide scale use of natural antifeedants is their availability. While crude neem extracts are readily available, this is not the case for the active constituent, azadirachtin, or for the great many antifeedants already recorded. Until natural antifeedants can be produced and extracted in a cost effective manner their use as applied agents will be limited. Research into biotechnological and synthetic routes for these materials offers hope in this respect. Particularly pertinent is the research attempting the total synthesis of azadirachtin (Ley 1990). It has been shown that a readily synthesised tricyclic furan compound that forms part of the azadirachtin structure, is as active to *Spodoptera littoralis* as azadirachtin itself (Blaney *et al.* 1990). The synthesis of simpler, yet active, antifeedants will allow new pest control strategies to be developed.

The second way in which antifeedants can be used is to consider them as transferable gene products that can be manipulated to develop host-plant resistance through genetic engineering. While it has not yet been demonstrated that the genes for complete biosynthetic pathways can be transferred from plant to plant, the transfer of genes that modify existing biosynthetic pathways in a plant has been accomplished. Examples of recently cloned and expressed plant genes include, the transfer of hyoscyamine hydroxylase gene from henbane into belladonna to express scopolamine (Matsuda *et al.* 1991) and the transfer of the stibene synthase gene from peanut and/or grapevine into tobacco to express the antifungal compound, resveratrol (Hain *et al.* 1991). The regenerated tobacco plants expressing the stibene synthase gene, showed increased resistance to infection by *Botrytis cinerea* (Hain *et al.* 1993). This is the first report of increased disease resistance in transgenic plants based on addition of a foreign secondary plant metabolite and opens up the possibility of determining pest resistance by moving a bioactive compound from one plant to another.

We have shown (Lane *et al.* 1985) that complex prenylated isoflavonoids are much more active antifeedants toward the grass grub larvae than unprenylated isoflavonoids such as medicarpin which is found in white clover (*Trifolium repens*), a favoured grass grub host-plant. As the first step towards introducing the genetic capability for the biosynthesis of prenylated isoflavonoids into white clover, a prenyltransferase enzyme from bean has been investigated (Biggs *et al.* 1990) but purification of this

membrane-bound enzyme has proved elusive to date. The tractability of biosynthetic enzymes and genes becomes a prime consideration in evaluating new candidate structures in relation to the modification of biosynthetic pathways in host plants by specific gene transfer.

Transgenic crop plants containing resistance genes offer hope for future pest control programmes but, at present, such transgenic plants contain only a single gene encoding for the protein toxin from *Bacillus thuringiensis*. The use of such plants will pose a strong selection pressure on pest insects and it is inevitable that insect resistance will develop. Plants counteract insect resistance evolution by employing a large number of defensive chemicals, usually antifeedants and/or repellents and usually in multicomponent mixtures and at variable concentrations. If transgenic plants are to be developed, chemical diversity needs to be introduced and this can only be accomplished through the knowledge of the chemical, biochemical and ecological interactions between plants and insects. In this respect, the study of insect antifeedants from plants, is likely to present us with exciting opportunities for future development of host-plant resistance.

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