ANTIFEEDANT AND INSECTICIDAL ACTIVITY OF SOME NEW ZEALAND GYMNOSPERMS.

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Many plants have chemical barriers that prevent them from acting as hosts to all but a few insect species. Some of these compounds, or their derivatives, will become the insect control agents of the future. Because of their origins and modes of action, it is possible that they could be more easily degradable, more species specific and less susceptible to the development of resistance than present synthetic pesticides. New Zealand flora, having being isolated since the Cretaceous period, may have evolved chemical barriers not found elsewhere in the world and provide a rich area for exploration for potential control agents. New Zealand has 19 endemic species and one endemic variety of gymnosperms (Connor and Edgar 1987). Extracts of several species inhibit insect moulting (Russell and Fenemore 1970) and a series of phytoecdysones have been identified in *Lepidothamnus intermedius* (Russell *et al.* 1972; Russell and Fraser 1973). Known feeding toxins exist in *L. intermedius* and *L. laxifolius*, three *Podocarpus* species, in *Phyllocladus trichmanoides* and in *Libocedrus bidwillii* (Singh *et al.* 1978). This paper reports the results of bioassays screening foliage extracts of some New Zealand gymnosperms for insect active compounds against two horticultural pests, two-spotted spider mite (TSM) *Tetranychus urticae*, and lightbrown apple moth (LBAM) *Epiphyas postvittana,* and two textile pests, Australian carpet beetle (ACB) *Anthrenocerus australis*, and webbing clothes moth (WCB) *Tineola bisselliella*.

Samples of foliage were collected from Dunedin Botanical Gardens in October 1992. Voucher specimens are kept in the Plant Extracts Research Unit collection at Otago University. Samples (5 g) after air-drying at 35°C and grinding, were extracted with 95% ethanol (50 ml) by shaking for 24 h. Extracts were filtered through cotton wool and stored at -15°C in air-tight bottles. Subsamples were used without further manipulation against WCM and ACB larvae. For the LBAM and TSM assays, subsamples (5 ml) were dried then redissolved in 95% ethanol (1 ml) to give a five-fold concentration.

The bioassay method used for late instar WCM and ACB larvae was as described by Gerard *et al.* (1992). Each cloth square was treated with 200 µl of the appropriate plant extract. Controls were either left untreated or treated with ethanol.

Antifeedant activity against LBAM larvae was assessed by cutting freeze-dried discs (18 mm diameter, mean weight 70 mg) of all purpose artificial diet (Singh 1983) into four quarters, treating two with 30 µl extract using a micropipette and the other two with 95% ethanol. Three replicates were prepared for each treatment. The four disc quarters were arranged at random in a petri dish, left at ambient temperatures overnight to allow solvent evaporation, rehydrated with 30 µl water/quarter then four 3rd instar LBAM larvae were introduced into each dish. After 48 hours at 20°C, each disc quarter was scored for feeding on a scale of 0-100 and the differences in the mean feeding score between the extract and control in each petri dish calculated. This experiment was seeking gross effects and would not detect small differences that may have been significant with greater replication.

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Toxicity to neonate LBAM larvae was determined by impregnating a freeze-dried artificial diet disc (18 mm diameter, 1.1 mm thick) with 120 µl of extract or 95% ethanol as a control. These were dried and rehydrated with 120 µl of water as above. Three replicates each consisting of a single disc with 30-40 neonate larvae were prepared. Larval survival was assessed after 1 and 3 days.

Phytoecdysone activity of extracts against LBAM was tested by topical application. Subsample extracts (200 µl) were dried, redissolved in 200 µl acetone and then applied with a microapplicator between the 3rd and 4th abdominal segments of 4th instar larvae at the rate of 0.75 µl/larva. There were three replicates of five larvae/treatment. After treatment, larvae were placed in individual plastic vials (10 ml) with c. 2 g artificial diet and maintained at 20°C until adult emergence. Larval mortality, pupal abnormalities and adult emergence were then assessed.

Contact toxicity against TSM was assayed by placing a strip of double sided sticky tape (9 mm x 20 mm) on a glass microscope slide. Ten µl of extract was smeared on the surface with disposable waxed card. Once dry, 25-50 adult females were placed dorsal side down on the tape surface using a fine brush. There were three replicates of each extract and untreated control. Mite mortality was assessed after 24 h at ambient temperatures, .

All bioassay data were analysed by ANOVA with LBAM and TSM percentages being subject to arcsin transformation.

TABLE 1: Survival, antifeedant and insect moulting responses of four insect species to gymnosperm extracts.

Plant species	Webbing clothes moth	Australian carpet beetle	Lightbrown apple moth	Two-spotted spider mite
Silver pine (*Lagarostrobos colensoi*)	-	FM	S	-
Pigmy pine (*Lepidothamnus laxifolius*)	SFM	FM	S	-
Kawaka (*Libocedrus plumosa*)	-	F	-	NT
Mountain toatoa (*Phyllocladus aspleniifolius* var. *alpinus*)	-	SFM	F	-
Toatoa (*Phyllocladus glaucus*)	-	F	-	S
Tanekaha (*Phyllocladus trichomanoides*)	SF	SFM	-	-
(*Podocarpus acutifolius*)	-	-	F	S

F = antifeedant activity significant (P<0.05)
S = survival reduction significant (P<0.05)
M = moulting interference significant (P<0.05)
- = no significant activity detected
NT = not tested

There was a wide variation in insect response (Table 1). *L. laxifolius* had greatest activity against ACB and the lepidopteran species, showing insecticidal, antifeedant and hormonal effects. Both WCM and ACB larvae suffered a low but significant mortality (12%) and had a 77% and 54% reduction in cloth consumption respectively on extracts of this plant. Mortality of LBAM on diet with *L. laxifolius* was 30% after 3 days, and extracts produced abnormal moulting in all species tested for this response, ranging from 14% (WCM, incomplete moult of exuviae) to one of the 15 LBAM larvae retaining larval legs at pupation. These results support the findings of Singh *et al.* (1978) who found high mortalities in codling moth (*Cydia pomonella*) and LBAM when reared from eggs to eclosion on diet containing milled leaf powder of this species and related this to the high concentration (1%) of phytoecdysones present. Although

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Singh *et al.* (1978) found marked insecticidal activity against codling moth larvae in all four *Podocarpus* species, activity was only found for *P. acutifolius* in the much shorter bioassays reported here. This species reduced LBAM feeding by 26% and increased TSM mortality by 20% compared with controls. *P. trichomanoides* showed insecticidal and antifeedant activity against both textile pests but not against the other two species. Visual inspection of the *P. trichomanoides* cloth samples during the bioassay revealed minimal feeding activity until the second week indicating the active compounds were not persistent. Singh *et al.* (1978) also found that extracts from this plant, which contain the phytoecdysone ponasterone A, have activity against some Lepidoptera but not LBAM.

The extract of *L. plumosa*, which contains the cytotoxic lignan, beta-peltatin (Perry and Foster unpublished results), showed antifeedant activity against ACB. Russell *et al.* (1976) found that two closely-related lignans caused the insecticidal activity of *L. bidwillii* extracts to codling moth and housefly (*Musca domestica*) larvae.

The extract of *L. colensoi* increased mortality of LBAM to 20% after 1 day and had an antifeedant and moulting effect on ACB. Singh *et al.* (1978) also detected insecticidal activity by this species, but the active component has not been identified (Russell, personal communication).

Kauri (*Agathis australis*), kahikatea (*Dacrycarpus dacrydioides*), rimu (*Dacrydium cupressinum*), Hall's totara (*Podocarpus hallii*), totara (*Podocarpus totara*), (*Podocarpus nivalis*) and ethanol had no effect on any of the insect species in the bioassays used.

The variation in insect response to gymnosperm extracts in our bioassays and those of Singh *et al.* (1978) demonstrates the necessity of using a range of insect species and tests to screen plant extracts for potentially active compounds. The concentration of active components in the extracts is likely to be very low and detection depends on the sensitivity of the bioassay and the insect species used. The longer feeding bioassays appear to provide more opportunity for detection of insecticidal, antifeedant and some moulting inhibition properties of extracts than bioassays of short duration. However, short duration bioassays are preferred to guide isolation of the bioactive components from plant extracts, which is the ultimate aim of this research.

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