INSECTICIDAL PROPERTIES OF THE TERPENOID POLYGODIAL, 9-DEOXYMUZIGADIAL AND AZADIRACHTIN.

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SUMMARY

Serial dilutions of plant extracts containing known concentrations of the terpenoid antifeedants, polygodial, 9-deoxymuzigadial and azadirachtin, were screened on woollen cloth against the webbing clothes moth (*Tineola bisselliella*) and the Australian carpet beetle (*Anthrenocerus australis*). Polygodial and 9-deoxymuzigadial showed insecticidal and antifeedant activity against webbing clothes moth and antifeedant activity against Australian carpet beetle at rates from 0.04 to 0.3% wool weight. Azadirachtin showed antifeedant activity against Australian carpet beetle, webbing clothes moth and the casemaking clothes moth (*Tinea dubiella*) at rates from 0.01 to 0.26% wool weight.

Keywords: polygodial, 9-deoxymuzigadial, azadirachtin, wool, insects, antifeedant

INTRODUCTION

Animal products such as wool, fur, feathers and horn can be damaged by insects that can feed on keratinaceous materials. Wool textiles requiring long term protection from these insects are treated with synthetic agents. In recent studies of alternative controls, a number of indigenous plant species with repellent properties were identified (Gerard and Ruf 1991) and the most active extracts screened for insecticidal or antifeedant activity against the casemaking clothes moth, *Tinea dubiella*. This indicated that horopito (*Pseudowintera colorata* (Family Winteraceae)) was highly active and warranted further investigation (unpublished data).

The essential oil of horopito is known to contain the bicyclic sesquiterpene dialdehyde, polygodial (McCallion *et al* 1982) which occurs in several plant genera. This compound has potent antifeedant activity against a number of lepidopteran species (e.g. Kubo *et al* 1976; Schoonhoven and Fu-Shun 1989) and inhibits aphid feeding, colonisation and transmission of some plant viruses (e.g. Gibson *et al*, 1982; Dawson *et al* 1986; Pickett *et al* 1987; Asakawa *et al* 1988; Griffiths *et al* 1989).

The leaves of the Indian neem tree (*Azadirachta indica* (Meliaceae)) have been used in India to keep insects away from clothes and grains (Jotwani and Srivastava 1984). The active principle, azadirachtin, inhibits feeding in a wide range of insects, often at very low rates, eg 0.04 ppm for the locust *Schistocerca gregaria* (Butterworth and Morgan 1971).

This paper reports on an investigation into the effectiveness of the active compounds from horopito and the Indian neem tree against the webbing clothes moth (*Tineola bisselliella*) and Australian carpet beetle (*Anthrenocerus australis*) larvae. Azadirachtin was also tested against the casemaking clothes moth.

MATERIALS AND METHODS

Cultures of insects are continuously reared under controlled environmental conditions. All compounds were tested against late instar larvae. The bioassay method was based on the International Wool Secretariat (IWS) test method No. 25 for assaying insect resistance (Anon 1984). Squares of untreated wool cloth (2.5 cm², standard abradant fabric, mean weight 164 mg) were placed in plastic petri dishes and 0.1 ml of treatment solution applied to each such that saturation occurred. Controls were

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cloth squares either left untreated or treated with solvent. Additional squares were treated with 0.1 ml of the solvent used in the treatment solution and were used to measure changes in cloth moisture content during the bioassay. Each treated cloth square was left to dry overnight at ambient temperatures then weighed and placed in an individually labelled 150 ml plastic pottle with well ventilated lid. Fifteen larvae were placed onto each of four replicates of the control and treated cloth. The pottles were held at 25°C and 50-60% relative humidity for 14 days. Then larval survival (i.e. active larvae) and wool weight loss (ie amount consumed) were assessed. The wool weight loss was corrected for moisture content using the weight changes observed in the moisture content controls.

Leaves of horopito were air dried and ground before sequential extraction with ethyl acetate, followed by ethanol, and then water. These extracts were assayed against the webbing clothes moth and the Australian carpet beetle and the ethyl acetate extract was found to be highly active (unpublished data). This extract was then separated by silica gel chromatography into 11 fractions which were subjected to the same bioassays and the most active fraction identified. This fraction was found to contain two closely related dialdehydes which were able to be separated by column chromatography using AgNO₃-impregnated silica gel. The first was polygodial and the other 9-deoxymuzigadial (unpublished data). Five sequential dilutions were made of each of these two dialdehydes in ethyl acetate to give rates ranging from 0.02 to 0.30% wool weight (ww) (Table 1).

A commercially available purified extract of neem seeds (Green Gold One, Entomological Workshop Pty Ltd, Australia) containing 1% azadirachtin was serially diluted with methylated spirits to give six rates ranging from 0.01 to 0.26% ww (Table 2).

All rates of the horopito compounds and azadirachtin were bioassayed simultaneously against each insect species. Concurrent tests were undertaken to determine survival of larvae after 14 days with and without food. Larvae were held either in empty test containers or with an untreated wool fabric square.

RESULTS

The results of the bioassays against the horopito compounds are presented in Table 1. There were no significant differences in activity of the two horopito dialdehydes ($P=0.08$). Both showed significant rate effects for cloth consumed by larvae ($P<0.001$) with rates down to 0.08% ww passing the IWS standards for insect resistance (little or no detectable damage and wool weight loss less than 15 mg). Significant treatment rate effects were found for larval survival of webbing clothes moth ($P<0.001$) with increasing concentrations resulting in decreased survival. There was little effect on Australian carpet beetle survival.

All rates of azadirachtin passed the IWS test for insect resistance for Australian carpet beetle and casemaking clothes moth but only the two highest rates passed the test for webbing clothes moth. There was a significant rate response for the cloth consumed ($P<0.001$) and survival ($P<0.001$) of larvae of webbing clothes moth and casemaking clothes moth. Increasing rates of azadirachtin decreased cloth consumption by Australian carpet beetle larvae ($P<0.001$) but had little effect on survival (Table 2).

The starvation tests showed a significantly lower percentage of starved larvae survived compared to fed larvae for webbing clothes moth (78.8 ± 5.6 versus 98.3 ± 1.5 , $P<0.05$) and the casemaking clothes moth (72.3 ± 5.6 versus 93.1 ± 3.5 , $P<0.05$) but no significant difference for Australian carpet beetle (87.9 ± 8.2 versus 100).

DISCUSSION

All three terpenoids, at application rates comparable to current commercial levels, reduced insect feeding damage on woollen cloth to below accepted IWS limits for insect resistance. Differences in survival of webbing clothes moth larvae exposed to the higher rates of the horopito terpenoids and the untreated controls far exceeded the approximate 20% difference found in the starvation test, indicating these compounds have insecticidal as well as antifeedant properties.

The horopito terpenoids may prove too unstable or toxic for the long term

TABLE 1: Effect of polygodial and 9-deoxymuzigadial on cloth consumption and survival of Australian carpet beetle (Acb) and webbing clothes moth (wcm) larvae

Treatment rate(%ww)	Mean cloth consumption (mg)		% survival±SE	
	Acb	wcm	Acb	wcm
polygodial				
0.30	3.9	-0.6	95±3	0
0.15	9.4	2.1	98±2	9±4
0.08	14.8	2.4	98±2	15±5
0.04	19.0	10.3	98±2	55±7
0.02	41.1	15.7	100	97±3
9-deoxymuzigadial				
0.30	2.5	-0.6	98±2	2±2
0.15	4.0	0.9	97±2	2±2
0.08	9.1	0.8	95±3	0
0.04	24.1	15.6	100	84±5
0.02	30.1	22.5	100	96±3
untreated	39.2	22.2	100	96±3
ethyl acetate	41.6	20.0	100	98±2
SED	4.3	1.9		

TABLE 2: Effect of azadirachtin on cloth consumption and survival of Australian carpet beetle (Acb), casemaking clothes moth (ccm) and webbing clothes moth (wcm) larvae

Rate (%ww)	Mean cloth consumed (mg)			% survival±SE		
	Acb	ccm	wcm	Acb	ccm	wcm
0.26	2.6	9.2	7.9	61±7	71±6	30±8
0.13	2.7	9.4	7.6	68±7	74±6	20±7
0.07	3.4	10.9	14.7	67±7	69±6	38±7
0.03	4.4	10.3	21.1	68±6	90±4	59±7
0.02	4.8	10.3	23.2	71±6	93±3	52±7
0.01	6.7	11.2	25.2	63±7	97±2	78±6
untreated	14.5	28.9	51.9	75±6	100	98±2
methylated spirits	15.3	26.8	49.5	73±6	97±2	98±2
SED	2.0	1.7	4.1			

protection of woollen textiles but azadirachtin could be an environmentally acceptable alternative to current treatments. A review by van Beek and de Groot (1986) stated that this compound is non-toxic to vertebrates (no rat mortality at oral doses of 8500 mg/kg), has no dermal toxicity, and is biodegradable.

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