

Biological Activity of Extracts of *Trichilia* Species and the Limonoid Hirtin Against Lepidopteran Larvae

Y. S. XIE,* M. B. ISMAN,*† P. GUNNING,* S. MACKINNON,‡ J. T. ARNASON,‡
D. R. TAYLOR,§ P. SANCHEZ,|| C. HASBUN† and G. H. N. TOWERS*

*Departments of Plant Science and Botany, University of British Columbia, Vancouver, BC, Canada V6T 1Z4;

‡Departments of Chemistry and Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5;

§Kwantlen College, P.O. Box 9030, Surrey, BC, Canada V3T 5H8;

||Museo Nacional, San Jose, Costa Rica;

†Departamento de Quimica, Universidad Nacional, Heredia, Costa Rica

Key Word Index—*Trichilia*; *T. connaroides*; hirtin; *Peridroma saucia*; *Spodoptera litura*; insecticidal activity; mode-of-action.

Abstract—Crude extracts of several *Trichilia* species (Meliaceae) were evaluated for their behavioral and physiological effects on the polyphagous lepidopterans *Peridroma saucia* and *Spodoptera litura*. A majority of extracts of nine *Trichilia* species, collected primarily from Costa Rica, significantly deter larval growth of *P. saucia*. Extracts of wood and bark are generally more active than those of foliage. Effects of various tissue extracts from *T. connaroides* on *P. saucia* and *S. litura* were comparatively studied. Extracts of bark, the most active tissue compared to those of wood, seed, exocarp and leaf, reduced larval growth of *P. saucia* and *S. litura* by 50% at dietary concentrations of 29.1 and 185.1 ppm, respectively. The limonoid hirtin, a constituent of *Trichilia hirta*, significantly inhibited larval growth of *P. saucia* in a dose-dependent manner, with a dietary EC₅₀ (effective concentration to inhibit growth by 50% relative to controls) of 13.0 ppm. Hirtin and various extracts from *T. connaroides* significantly reduced larval growth and consumption rates, as well as dietary utilization, indicating that these substances possess both behavioral effects and post-ingestive toxicity.

Introduction

In a recent review of botanical insecticides, Jacobson (1989) pointed out that “the most promising botanicals for use at the present and in the future are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae.” In the exploration for insecticides of plant origin, the Meliaceae has attracted extensive attention (Arnason *et al.*, 1993; Isman *et al.*, 1993). The biological activity of limonoids from the plant order Rurales, to which the Meliaceae belongs, has also been reviewed (Champagne *et al.*, 1992). It is well documented that the limonoid azadirachtin, the active principle in seeds of the neem tree, *Azadirachta indica* (Meliaceae), has toxic, antifeedant and insect growth regulating activities, and over 300 species of insects and mites are known to be susceptible to the action of neem (Schmutterer, 1990, 1993). Some neem-based commercial products have been developed and approved for use in the United States (Walter and Knauss, 1990; Wood, 1990).

However, the extreme structural complexity of the azadirachtin molecule makes it unlikely that chemical synthesis can be achieved on a commercial scale, in spite of considerable synthetic work (Simmonds *et al.*, 1990). It seems likely that neem-based commercial products will depend on the ongoing availability of neem seed. Several investigators have been searching for other plant species which may provide alternative plant sources to neem or may have simpler phytochemicals more amenable to synthesis for pest control. The genus *Trichilia* (Meliaceae) appears to have potential in that regard. For example, a series of limonoids, with the trivial name trichilins, have been isolated from *T. roka* and shown to be antifeedants for the

†Author to whom correspondence should be addressed.

southern armyworm (*Spodoptera eridania*) and the Mexican bean beetle (*Epilachna varivestis*) (Nakatani *et al.*, 1981, 1985). The apo-euphol limonoid sendanin has been identified in the fresh fruit of *T. roka* and shown to have growth inhibitory effects on four lepidopteran pests of cotton (Kubo and Klocke, 1982). Ethanol extracts of seeds of *T. prieureana*, *T. roka* and *T. connaroides* exhibited high levels of activity in both choice and no-choice bioassays against *S. frugiperda* (Mikolajczak and Reed, 1987; Mikolajczak *et al.*, 1989). A number of limonoids from *T. hirta*, *T. havanensis*, *T. trifolia* and *T. prieureana* have been isolated (Chan and Taylor, 1966; Burke *et al.*, 1977; Cortez *et al.*, 1992; Chan *et al.*, 1973; Arenas and Rodriguez-Hahn, 1990; Taylor, 1971; Olugbade, 1991), but the bioactivity of limonoids from these species has not been explored.

The objectives of this study were to screen ethanol extracts from different *Trichilia* species for insecticidal activity, to characterize the bioactivity of the pure compound hirtin (Fig. 1) from *T. hirta* against the variegated cutworm, *Peridroma saucia* Hübner, and to investigate the proximate mode-of-action of hirtin and crude extracts of *T. connaroides* on both *P. saucia* and the tobacco caterpillar, *Spodoptera litura* Fabr.

Materials and Methods

Plant extracts. Twenty-two plant samples from nine different species of *Trichilia* were collected; eight species from Costa Rica and one (*T. connaroides*) from India. Voucher specimens have been retained by the University of Ottawa, Ottawa, Canada, the National Museum, San Jose, Costa Rica, and Dr C. Saldhana, St. Joseph's College, Bangalore, India. Freshly collected plant materials (ca 200 g) were extracted with 95% ethanol (3×300 ml) over 3 days. After filtration, the combined extracts were evaporated *in vacuo*, freeze dried and resuspended in methanol to afford the extracts for testing.

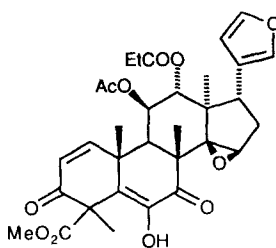
Hirtin was extracted from *T. hirta* as described in Chan and Taylor (1966). Purity was better than 95% based on high performance liquid chromatography (HPLC).

Insects. The test insects used in this study were obtained from laboratory cultures maintained at 21±1°C and a photoperiod of 16L:8D, and reared on artificial diet (BioServ Inc., Frenchtown, New Jersey).

Screening bioassays. Plant extracts were incorporated into artificial diet at a concentration of 0.2% fresh wt. of diet by the method of Isman and Rodriguez (1983). Control diets were treated with the carrier solvent (methanol) alone. Two neonate *P. saucia* larvae were placed in individual compartments in a plastic tray with approximately 1 g of treated or control diet (*N*=20). Larvae were maintained in a growth chamber at 25°C, and a photoperiod of 16L:8D. After 7 and 10 days, all larvae were individually weighed, and the mean weights for each treatment group were expressed as a percentage of controls.

Dose-response experiments. Pure hirtin and crude extracts from exocarp, seed, wood and bark of *T. connaroides* were subject to dose-response bioassays. Experimental diets contained hirtin at concentrations of 5, 10, 15, 20 or 25 ppm fresh wt. of diet, and plant extracts at concentrations of 200, 400, 600, 800, or 1000 ppm fresh wt. of diet. Bioassays were performed with neonate larvae as described above.

Diet choice test. In order to assess behavioral response of larvae of *P. saucia* and *S. litura*, a diet choice test was performed by placing four fresh diet cubes, two containing plant extracts at concentrations of 200, 400, 600, 800, or 1000 ppm fresh wt. and two controls treated with solvent alone, in a plastic Petri dish (5 cm dia.) (*N*=10). Twenty second-instar larvae were introduced into the centre of each Petri dish. After 24 h (25°C), the number of larvae on each diet type was recorded. The feeding deterency index (%) for each treatment was calculated as $(C-T)/(C+T) \times 100$, where C is the number of larvae on control diets and T is the number of larvae on treated diets (Isman *et al.*, 1990).



Hirtin

FIG. 1. THE STRUCTURE OF THE LIMONOID HIRTIN.

Nutritional experiments. Nutritional experiments were conducted in order to separate behavioral responses from toxic effects. For hirtin, nutritional experiments were performed in two different ways, dietary feeding and topical application, using fourth-instar larvae of *P. saucia*. In the former case, 20 larvae were individually reared on an appropriate amount of artificial diet treated with hirtin at concentrations of 20, 30 or 40 ppm fresh wt. or solvent alone (control). In the latter case, 20 larvae of the same age as above were treated topically with hirtin at doses of 5, 10, or 15 $\mu\text{g larva}^{-1}$ in 1 μl of acetone (with appropriate solvent as control), and then allowed to feed on untreated diet for 3 days. For crude extracts from exocarp, seed, wood and bark of *T. connaroides*, test materials were incorporated into artificial diet at a concentration of 500 ppm fresh wt. of diet. All nutritional indices were calculated on the basis of dry wts, and therefore, at the outset of experiments, 10 diet samples (ca 0.5 g each) and 20 individual larvae were taken and dried to constant weight at 60°C to determine fresh wt./dry wt. ratios. After 3 days of feeding, dry wts were determined for remaining diet, feces and larvae. The following nutritional indices were calculated as previously described (Manuwoto and Scriber, 1982; Farrar and Kennedy, 1987; Farrar *et al.*, 1989): relative growth rate (RGR_r) = mg of biomass gained per mg of initial larval biomass per day; relative consumption rate (RCR_r) = mg of biomass ingested per mg of initial larval biomass per day; approximate digestibility (AD) = (food ingested - feces) / (food ingested) × 100; efficiency of conversion of digested food (ECD) = (biomass gained) / (food ingested - feces) × 100; efficiency of conversion of ingested food (ECI) = (biomass gained) / (food ingested) × 100.

Data analysis. Statistical software (Anonymous, 1991) was used for data analysis. A normality test and a homogeneity test were performed to determine if the data were parametric. Parametric analyses of variance (ANOVA) or nonparametric analysis of variance (Kruskal-Wallis test), as required, were performed for all data. When significant differences were found by analysis of variance, the least significant difference (LSD) test was applied for comparisons among the means. Linear regression analysis was applied for all dose-response experimental data (Anonymous, 1991).

Results

Screening bioassays

There were significant differences ($P < 0.05$) among the nine *Trichilia* species with respect to inhibition of larval growth of *P. saucia* by standardized extracts (Table 1), with *T. hirta* and *T. connaroides* being generally the most active. Within each species,

TABLE 1. GROWTH INHIBITORY EFFECT OF EXTRACTS OF *TRICHILIA* SPECIES ON *P. SAUCIA* AT A DIETARY CONCENTRATION OF 0.2% FRESH WT. OF DIET

Plant species	Component extracted	Larval weight (% of control)	
		Day 7	Day 10
<i>T. martiana</i> C.DC	Leaf	104.8 a*	86.2 b
<i>T. connaroides</i> (Wright & Am.)	Leaf	97.4 ab	82.2 b
<i>T. havanensis</i> Jacq.	Wood	95.7 bc	104.0 a
<i>T. havanensis</i> Jacq.	Leaf	90.9 b-d	64.3 c
<i>T. pleeana</i> (A. Juss.) C.DC	Leaf	88.9 cd	63.6 c
<i>T. havanensis</i> Jacq.	Bark	83.6 de	58.8 cd
<i>T. pleeana</i> (A. Juss.) C.DC	Wood	76.8 ef	66.7 c
<i>T. glabra</i> L.	Leaf	72.2 f	54.1 d
<i>T. americana</i> (Sesse & Mocino) Pennington	Leaf	60.6 g	43.2 e
<i>T. americana</i> (Sesse & Mocino) Pennington	Bark	57.6 g	24.8 f
<i>T. quadrijugata</i> subsp. <i>cinerascens</i> (C.DC) Pennington	Leaf	55.0 g	27.8 f
<i>T. pleeana</i> (A. Juss.) C.DC	Bark	23.9 h	8.3 g
<i>T. glabra</i> L.	Bark	18.4 hi	9.4 g
<i>T. hirta</i> L.	Leaf	17.5 hi	5.3 g
<i>T. trifolia</i> L.	Wood	13.4 ij	6.4 g
<i>T. connaroides</i> (Wright & Am.)	Exocarp	9.8 jk	—†
<i>T. hirta</i> L.	Bark	8.8 jk	—
<i>T. connaroides</i> (Wright & Am.)	Seed	5.1 jk	1.4 g
<i>T. connaroides</i> (Wright & Am.)	Wood	2.5 k	0.5 g
<i>T. connaroides</i> (Wright & Am.)	Bark	2.1 k	0.6 g
<i>T. glabra</i> L.	Wood	1.8 k	—
<i>T. hirta</i> L.	Wood	—	—

*Means followed by the same letters within columns indicate no significant difference ($P > 0.05$) in LSD test.

†All insects died before assessment.

crude extracts from different tissues also showed significant differences in bioactivity against this insect. For instance, the wood extract of *T. hirta* was the most active sample tested, with all insects dying before day 7, whereas larvae exposed to the foliar extract were all alive, but only 17.5% as heavy as control larvae at day 7. The crude extract of *T. connaroides* foliage was inactive against *P. saucia* (97.4% of controls at day 7), whereas the bark extract was very active (2.1% of controls) (Table 1). Generally, bark and wood were the most active components in the *Trichilia* species examined.

Dose-response experiments

When neonate *P. saucia* were fed on artificial diet containing hirtin at concentrations of 5–25 ppm for 7 days, larval growth was significantly reduced in a dose-dependent manner ($r = -0.95$, $P < 0.05$). After 7 and 10 days of feeding, the EC_{50} (effective concentration to inhibit growth by 50% relative to controls) was 13.0 ppm and 11.5 ppm, respectively. Larval growth of both *P. saucia* and *S. litura* was significantly and negatively correlated with dietary concentration of crude extracts (exocarp, seed, wood and bark) of *T. connaroides*. EC_{50} values generated by linear regression analysis are given in Table 2. Bark extract showed a significantly stronger ($P < 0.05$) effect on both insects compared with extracts of other tissues (exocarp, seed and wood) (Table 2). *Peridroma saucia* was more sensitive to the inhibitory effect of the extracts than *S. litura*.

Diet choice test

Second-instar larvae were used in diet choice tests because they were found to generate less variable results than neonate larvae (Xie and Isman, 1992). The number of insects on control diets was significantly correlated ($P < 0.05$) to dietary concentration of the crude extracts. DC_{50} (concentration resulting in a deterrency index of 50%) values generated by linear regression analysis are shown in Table 3. This result was

TABLE 2. GROWTH INHIBITORY EFFECT OF EXTRACTS OF *T. CONNAROIDES* ON NEONATE *P. SAUCIA* AND *S. LITURA* LARVAE

Component extracted	<i>P. saucia</i> EC_{50} * (95% CI) (ppm)	<i>S. litura</i> EC_{50} * (95% CI) (ppm)
Exocarp	542.5 (494.6–590.3)	701.0 (648.3–753.8)
Seed	461.5 (417.9–505.1)	735.2 (674.6–795.7)
Wood	387.5 (331.6–443.4)	618.5 (585.5–651.4)
Bark	29.1 (5.5–60.1)	185.1 (117.8–252.3)

* EC_{50} = effective concentration to reduce larval growth by 50% relative to controls.

TABLE 3. DETERRENT EFFECT OF EXTRACTS OF *T. CONNAROIDES* ON SECOND INSTAR *P. SAUCIA* AND *S. LITURA* LARVAE

Component extracted	<i>P. saucia</i> DC_{50} * (95% CI) (ppm)	<i>S. litura</i> DC_{50} * (95% CI) (ppm)
Exocarp	> 1000	> 1000
Seed	693.2 (569.4–817.0)	852.7 (620.7–1084.8)
Wood	930.5 (831.9–1029.2)	832.8 (615.9–1049.6)
Bark	441.2 (273.9–608.4)	530.4 (357.6–703.1)

* DC_{50} = concentration resulting in a deterrency index of 50%. See text for calculation of deterrency index.

consistent with that from dose-response experiments, in that bark was the most active of the tissues with respect to antifeedant action.

Nutritional experiments

Hirtin incorporated into artificial diet at all concentrations tested (20, 30 and 40 ppm) and topically applied to larvae at all doses (5, 10 and 15 µg insect⁻¹) significantly ($P < 0.05$) reduced the relative growth rate (RGR_i) and relative consumption rate (RCR_i) of *P. saucia* larvae (Table 4), compared to controls. These reductions were significantly ($P < 0.05$) correlated with dietary concentrations or topical doses based on linear regression. The efficiencies of conversion of ingested and digested food (ECI and ECD) into larval biomass were significantly reduced at dietary concentrations ≥ 30 ppm, or at topical doses ≥ 10 µg insect⁻¹. Approximate digestibility (AD), however, remained unaffected in all treatments except at the higher dietary concentrations (30 and 40 ppm) where it increased (Table 4).

At a concentration of 500 ppm fresh wt. of diet, all four extracts (exocarp, seed, wood and bark) of *T. connaroides* significantly ($P < 0.05$) reduced RGR_i, RCR_i, ECI and ECD for both *P. saucia* and *S. litura* (except exocarp on ECI and ECD) when compared with controls (Table 5).

TABLE 4. GROWTH, FEEDING AND DIETARY UTILIZATION BY *P. SAUCIA* AFTER FEEDING AND TOPICAL APPLICATION OF HIRTIN

Treatment	RGR _i (mg mg ⁻¹ day ⁻¹)	RCR _i (mg mg ⁻¹ day ⁻¹)	ECI (%)	ECD (%)	AD (%)
Dietary concentration (ppm)					
0	0.95*	3.23*	30.41*	92.72*	42.95
20	0.55	2.19	26.54	73.29	37.79
30	0.48	1.99	24.28	48.58	51.77
40	0.33	1.64	20.77	42.30	54.53
Topical applied dose (µg insect ⁻¹)					
0	2.50*	7.10*	37.39*	90.67*	46.33
5	1.78	5.03	35.51	83.87	43.95
10	1.30	4.33	29.61	52.22	39.66
15	0.90	3.65	26.19	50.54	38.15

Abbreviations: RGR_i = relative growth rate; RCR_i = relative consumption rate; AD = approximate digestibility; ECD = efficiency of conversion of digested food; ECI = efficiency of conversion of ingested food.

*Indicates significant linear regression ($P < 0.05$) within column.

TABLE 5. GROWTH, FEEDING, AND DIETARY UTILIZATION BY *P. SAUCIA* AND *S. LITURA* AFTER FEEDING ON ARTIFICIAL DIET TREATED WITH *T. CONNAROIDES* EXTRACTS AT A CONCENTRATION OF 500 PPM FRESH WT.

Treatment	RGR _i (mg mg ⁻¹ day ⁻¹)	RCR _i (mg mg ⁻¹ day ⁻¹)	ECI (%)	ECD (%)	AD (%)
<i>P. saucia</i>					
Control	1.6 a*	4.5 a	42.9 a	90.1 a	49.9 a
Exocarp	1.0 b	2.6 b	33.8 b	74.1 b	60.3 a
Seed	0.7 c	2.3 b	33.9 b	71.4 b	51.6 a
Wood	0.5 d	1.7 c	30.1 c	57.3 c	57.1 a
Bark	0.5 d	1.8 c	28.2 c	51.6 c	59.6 a
<i>S. litura</i>					
Control	2.2 a	7.7 a	28.2 a	43.5 a	69.8 a
Exocarp	1.6 b	5.9 b	27.3 a	42.1 a	69.2 a
Seed	1.5 b	5.7 b	25.9 b	37.8 b	69.1 a
Wood	1.1 c	4.8 c	22.8 c	32.8 c	65.2 b
Bark	0.7 d	4.3 d	16.6 d	24.4 d	65.1 b

Abbreviations are the same as in Table 4.

*Means followed by the same letters within columns indicate no significant difference ($P > 0.05$) in LSD test.

Discussion

Intensive biotic interactions in the tropics and subtropics have led to the evolution of a great diversity of secondary metabolites in terrestrial plants. These chemicals are frequently found to have behavioral or toxic action, or both, to phytophagous insects (Arnason *et al.*, 1989). Therefore, it is not surprising to find that half of our *Trichilia* samples collected from these regions are biologically active against our test insect, reducing *P. saucia* larval weight to <30% of controls at a dietary concentration of 0.2% fresh wt. (Table 1). Comparison of the bioactivity of extracts from different plant tissues indicates that wood and bark of *Trichilia* species are generally more active than foliar extracts in our bioassays. This result is similar to that obtained with species of *Cedrela* tested against the insect *Ostrinia nubilalis* (Arnason *et al.*, 1993). Differing chemical profiles of the tissues and organs suggest an evolutionary advantage: foliage loss is cheap compared to bark or wood loss in terms of replacement.

Although hirtin was isolated and identified in the mid-1960s, its bioactivity to insects was previously unknown. Our results indicate that it has significant growth inhibitory action (*P. saucia* EC_{50} = 13.0 ppm), being about four times more active than cedrelone, a limonoid isolated from *Toona ciliata* (Meliaceae) against the same insect (Koul and Isman, 1992). Hirtin was also found to be quite active against another noctuid, the zebra caterpillar (*Melanchra picta*), with an EC_{50} of 21.1 ppm (unpublished data).

The bioactivities of hirtin against insects were further demonstrated from the two nutritional experiments, wherein both oral (in artificial diet) and topical administration resulted in significant reductions ($P < 0.05$) in RGR, and RCR_i , with concomitant reductions in ECI and ECD (Table 4). Parallel results were also obtained with *M. picta* (unpublished data). These results confirmed that hirtin possesses both antifeedant and toxic effects against insects.

Initial screening of *Trichilia* extracts indicated that *T. connaroides* was very promising because all extracts from this species (except the foliar extract) possessed strong bioactivity against *P. saucia*. In particular, the bark extract is very active (*P. saucia* EC_{50} = 29.1 ppm) and even more active than pure cedrelone (*P. saucia* EC_{50} = 52.0 ppm) (Koul and Isman, 1992).

Choice tests are useful in detecting small differences in food acceptability (Schoonhoven, 1982), and are widely used to determine behavioral effects of test materials on insects. The diet choice bioassay in our study clearly demonstrated that when crude extracts of *T. connaroides* were incorporated into artificial diet, the food acceptability of both insect species was reduced in a dose-dependent manner, suggesting that chemsensory effects may be involved. These behavioral effects were also corroborated by the nutritional experiments, which showed significant reduction ($P < 0.05$) in RCR_i for both insect species in response to the test substances compared to controls (Table 5).

Nutritional experiments differentiate between inhibition of growth attributable to behavioral effects (antifeedant action) and that resulting from post-ingestive toxicity. The present study of nutritional analyses following oral administration (in artificial diet) of *T. connaroides* extracts to *P. saucia* and *S. litura* revealed significant reductions ($P < 0.05$) in both growth and consumption rate with concomitant significant reductions ($P < 0.05$) in dietary utilizations (ECI and ECD) (Table 5). These results confirm both behavioral effects and post-ingestive toxicity as modes-of-action resulting in suppression of larval growth by *T. connaroides* extracts. As well, significant inhibition ($P < 0.05$) of AD with wood and bark extracts to *S. litura* (Table 5) could result from a direct effect on digestive enzymes of this insect.

We believe that hirtin is responsible for much, if not most, of the bioactivity of *T. hirta*, a very active species in our screening program. However, our primary phytochemical analysis (by HPLC) revealed that hirtin is only a trace constituent in *T.*

connaroides extracts, and likely does not account for the strong bioactivity. Isolation and identification of the active compounds in *T. connaroides* are under investigation.

Acknowledgements—We gratefully thank Yanfen Zheng for technical assistance. Supported by a strategic grant from the Natural Sciences and Engineering Research Council of Canada.

References

- Anonymous (1991) *Statistix (3.5) User's Manual*. Analytical Software. St. Paul, MN.
- Arenas, C. and Rodriguez-Hahn, L. (1990) Limonoids from *Trichilia havanensis*. *Phytochemistry* **29**, 2953–2956.
- Arnason, J. T., MacKinnon, S., Durst, A., Philogène, B. J. R., Hasbun, C., Sanchez, P., Poveda, L., Roman, L. S., Isman, M. B., Satasook, C., Towers, G. H. N., Wiriyachitra, P. and McLaughlin, J. L. (1993) Insecticides in tropical plants with non-neurotoxic modes of action. In *Phytochemical Potential of Tropical Plants* (Downum, K. R., Romeo, J. T. and Stafford, H. A. P., eds), pp. 107–131. *Rec. Adv. Phytochem.* **27**, Plenum Press, New York.
- Arnason, J. T., Philogène, B. J. R. and Morand, P. (eds) (1989) *Insecticides of Plant Origin*. *Am. Chem. Soc. Symp. Ser.* **387**, Washington, DC.
- Burke, B. A., Chan, W. R., Rawle, J. R. and Taylor, D. R. (1977) Correlation of anthothecol and hirtin. *Experientia* **33**, 578–579.
- Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N. (1992) Biological activity of limonoids from the Rutales. *Phytochemistry* **31**, 377–394.
- Chan, W. R., Gibbs, J. A. and Taylor, D. R. (1973) Triterpenoids from *Trichilia havanensis* Jacq. Part I. The Acetates of Havanensin and Trichilenone, New Tetracarboxylic Tetranortriterpenes. *J.C.S. Perkin I*, 1047–1050.
- Chan, W. R. and Taylor, D. R. (1966) Hirtin and deacetylhirtin: New “limonoids” from *Trichilia hirta*. *J. Chem. Soc. Chem. Commun.* 206–207.
- Cortez, D. A. G., Vieira, P. C., Fernandes, J. B., Fatima, M., de Silva, G. F. and Ferreira, A. G. (1992) Limonoids from *Trichilia hirta*. *Phytochemistry* **31**, 625–628.
- Farrar, R. R., Jr., Barbour, J. D. and Kennedy, G. G. (1989) Quantifying food consumption and growth in insects. *Ann. Ent. Soc. Am.* **82**, 593–598.
- Farrar, R. R. Jr. and Kennedy, G. G. (1987) Growth, food consumption and mortality of *Heliothis zea* larvae on foliage of the wild tomato *Lycopersicon hirsutum* and the cultivated tomato *L. esculentum*. *Entomol. Exp. Appl.* **44**, 213–219.
- Isman, M. B., Arnason, J. T. and Towers, G. H. N. (1993) Plants of the family Meliaceae as potential sources of botanical insecticides. In *The Neem Tree—A Source of Unique Products for Pest Management and Other Purposes* (Schmutterer, H., ed.), VCH, Weinheim (in press).
- Isman, M. B., Koul, O., Luczynski, A. and Kaminski, J. (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *J. Agric. Food Chem.* **38**, 1406–1411.
- Isman, M. B. and Rodriguez, E. (1983) Larval growth inhibitors from species of *Parthenium* (Asteraceae). *Phytochemistry* **22**, 2709–2713.
- Jacobson, M. (1989) Botanical pesticides—past, present, and future. In *Insecticides of Plant Origin* (Arnason, J. T., Philogène, B. J. R. and Morand, P., eds), pp. 1–10. *Am. Chem. Soc. Symp. Ser.* **378**, Washington, DC.
- Koul, O. and Isman, M. B. (1992) Toxicity of the limonoid allelochemical cedrelone to noctuid larvae. *Entomol. Exp. Appl.* **64**, 281–287.
- Kubo, I. and Klocke, J. A. (1982) An insect growth inhibitor from *Trichilia roka* (Meliaceae). *Experimentia* **38**, 639–640.
- Manuwoto, S. and Scriber, J. M. (1982) Consumption and utilization of three maize genotypes by the southern armyworm. *J. Econ. Entomol.* **75**, 163–167.
- Mikolajczak, K. L. and Reed, D. K. (1987) Extractives of seeds of the Meliaceae: Effects on *Spodoptera frugiperda* (J. E. Smith), *Acalymma vittatum* (F.), and *Artemia salina* Leach. *J. Chem. Ecol.* **13**, 99–111.
- Mikolajczak, K. L., Zilkowski, B. W. and Bartelt, R. J. (1989) Effects of meliaceous seed extracts on growth and survival of *Spodoptera frugiperda* (J. E. Smith). *J. Chem. Ecol.* **15**, 121–128.
- Nakatani, M., Iwashita, T., Naoki, H. and Hase, T. (1985) Structure of a limonoid antifeedant from *Trichilia roka*. *Phytochemistry* **24**, 195–196.
- Nakatani, M., James, J. C. and Nakanishi, K. (1981) Isolation and structures of trichilins, antifeedants against the southern army worm. *J. Am. Chem. Soc.* **103**, 1228–1230.
- Olugbade, T. A. (1991) Tetracyclic triterpenoids from *Trichilia prieuriana* leaves. *Phytochemistry* **30**, 698–700.
- Schmutterer, H. (1990) Properties and potential natural pesticides from the neem tree. *A. Rev. Entomol.* **35**, 271–297.
- Schmutterer, H. (ed) (1993) *The Neem Tree—A Source of Unique Products for Pest Management and Other Purposes*. VCH, Weinheim (in press).
- Schoonhoven, L. M. (1982) Biological aspects of antifeedants. *Entomol. Exp. Appl.* **31**, 57–69.

- Simmonds, M. S. J., Blaney, W. M., Ley, S. V., Anderson, J. C. and Toogood, P. L. (1990) Azadirachtin: structural requirements for reducing growth and increasing mortality in lepidopterous larvae. *Entomol. Exp. Appl.* **55**, 169–181.
- Taylor, D. R. (1971) New limonoids from *Trichilia trifolia* (Meliaceae). *Rev. Latinoamer. Quim.* **2**, 87–92.
- Walter, J. F. and Knauss, J. F. (1990) Developing a neem-based pest management product. In *Neem's Potential in Pest Management Programs, Proceedings of the USDA Neem Workshop* (Locke, J. C. and Lawson, R. H., eds), pp. 29–31. United States Department of Agriculture, Agricultural Research Service, ARS-86, Beltsville, Maryland.
- Wood, T. (1990) Efficacy of neem extracts and neem derivatives against several agricultural insect pests. In *Neem's Potential in Pest Management Programs, Proceedings of the USDA Neem Workshop* (Locke, J. C. and Lawson, R. H., eds), pp. 76–84. United States Department of Agriculture, Agricultural Research Service, ARS-86, Beltsville, Maryland.
- Xie, Y. S. and Isman, M. B. (1992) Antifeedant and growth inhibitory effects of tall oil and derivatives against the variegated cutworm, *Peridroma saucia* Hübner (Lepidoptera: Noctuidae). *Can. Entomol.* **124**, 861–869.