Chapter Six

TROPICAL FORESTS AS SOURCES OF NATURAL INSECTICIDES

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INTRODUCTION

It is commonly held that tropical and subtropical plants are richer sources of defensive allelochemicals than their temperate zone counterparts because the pressure from insect herbivores, plant pathogens, and grazing mammals is substantially greater in tropical ecosystems. Indeed, sources of the botanical insecticides rotenone (*Derris* and *Lonchocarpus*, Leguminosae) and nicotine (*Nicotiana*, Solanaceae) are tropical in origin, but the most widely used botanical, pyrethrum (from *Tanacetum cinerariorum*, Asteraceae), originates from and grows best in more temperate climates. A number of factors together have provided the impetus for continued phytochemical exploration of tropical plants in pursuit of new botanical (naturally-sourced) insecticides or as agrochemical leads. These include: 1) the 'western discovery' of the profound insect control properties of extracts from the neem tree (*Azadirachta indica*, Meliaceae) and their commercialization as botanical insecticides in India, the U.S.A., and Germany; 2) the continuing belief that new pesticides and medicines with fewer detrimental health and environmental effects are yet to be discovered from plants; 3) rampant deforestation with resulting loss of biodiversity in Old and New World tropical rainforests; and 4) the hope that discovery of valuable non-timber forest products in tropical regions will produce competition for logging and justify forest conservation, particularly in developing countries.

Since the present subject was last reviewed over a decade ago,[1-5] numerous reports on the bioactivity of allelochemicals from tropical trees against insects have appeared, but with little commercial exploitation of the source species. In this chapter, I review some recent work on tropical trees from the families Meliaceae, Annonaceae, and Myrtaceae, each of which shows promising bioactivity against insects, and may be, at the least, suitable for indigenous use as crop protectants, if not for harvest, refinement, and export for crop protection in industrialized countries.

SPECIES OF MELIACEAE AS SOURCES OF NATURAL INSECTICIDES

There can be no doubt that interest in the mahogany family (Meliaceae) was spurred by the development of natural insecticides from the Indian neem tree, *Azadirachta indica* A. Juss. This species and its commercial utility has been the subject of several international conferences, hundreds of research papers, and at least a dozen major volumes.[6-7] This interest was manifest in the collection and screening of more than 100 species from the family for insect bioactivity, with particular emphasis on the genera *Aglaia* and *Trichilia*. Even before this, attention had turned to members of the genus most closely related to neem, *Melia*, namely the Asian chinaberry tree *M. azedarach* L. and the East African *M. volkensii* Gurke.

Melia volkensii

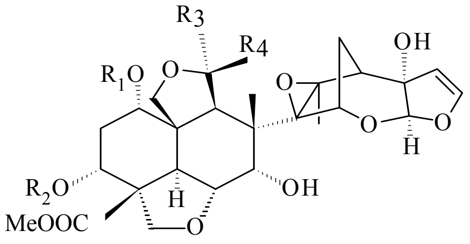
This species grows at moderate elevations in Kenya and adjoining east African countries. A crude fruit extract was first recognized as an antifeedant to desert locusts over twenty years ago,[8] but extracts have subsequently been shown to be toxic to or interfere with growth of a wide range of insect pests.[9] At least eight limonoids have been isolated from the fruits; although the putative active ingredients from neem seeds, the azadirachtins (**1,2**), do not occur in *M. volkensii*, other limonoids such as salannin occur in both species. A major and unique constituent, volkensin (**3**), is an effective antifeedant, but none of the compounds isolated shows insect growth regulatory (IGR) activity in the Mexican bean beetle, *Epilachna varivestis* (Fig. 6.1).

In our hands, a fruit extract of *M. volkensii* proved to be a potent antifeedant and larval growth inhibitor to both the cabbage looper, *Trichoplusia ni* (Noctuidae) and the armyworm, *Pseudaletia unipuncta* (Noctuidae).[10] However, unlike the armyworm, the looper is capable of habituating to the extract, *i.e.* feeding deterrence diminishes following continuous exposure.[11]

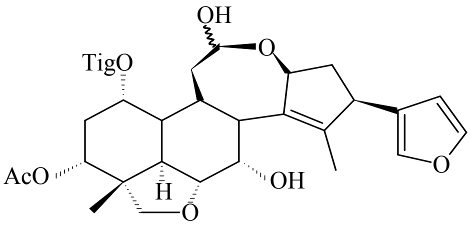
This fast-growing species begins to set fruit in 4-5 years, and unlike neem, produces fruit almost year-round, making it suitable for small-scale regional use in east Africa. On the other hand, the complex chemistry and dearth of proper toxicological data make it highly unlikely to be developed for use in tightly regulated countries.

Melia azedarach

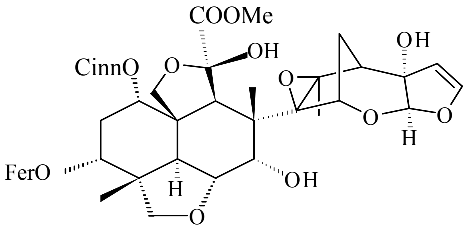
The chinaberry tree, native to eastern Asia, has been widely introduced and cultivated throughout the tropics and subtropics. Fruit extracts of *M. azedarach* have long been known to have insecticidal activities, in some cases comparable to that of neem.[12] Like *M. volkensii*, *M. azedarach* lacks azadirachtins, but fruits produce the chemically related meliacarpins (*e.g.* **4**) that have azadirachtin-like bioactivity in many types of insect.[13] Unlike neem, exploitation of chinaberry extracts for crop protection has been avoided owing to the presence of meliatoxins (*e.g.* **5**), limonoid constituents with demonstrated toxicity to mammals. However, fruit extracts from *M. azedarach* growing in Argentina lack toxicity in rats, and moreover, contain a unique limonoid, meliartenin (**6**), that has both antifeedant and insecticidal activities against a range of insects[14] (Fig. 6.1).



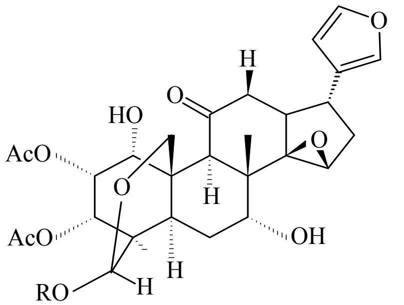
	R_1	R_2	R_3	R_4	
1	OTig	OAc	$COOCH_3$	OH	Azadirachtin
2	OH	OTig	$COOCH_3$	H	3-Tigloylazadirachtol ('Aza B')
8	OTig	OAc	H	OAc	Marrangin
9	OBenz	OAc	$COOCH_3$	OH	1-Benzoyl-1-detigloylazadirachtin
10	OH	OIsoval	$COOCH_3$	H	3-Isovaleroylazadirachtol



3 Volkensin

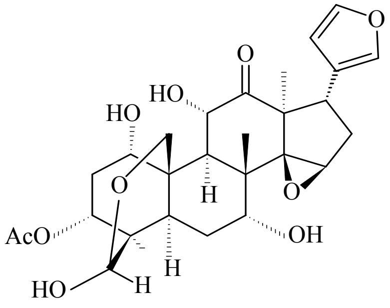


4 1-Cinnamoyl-3-feruoyl-11-hydroxymeliacarpin

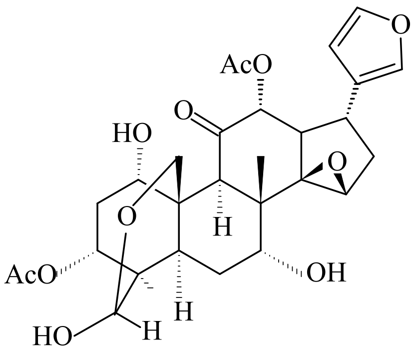


R = $MeCH_2CHMeCO$

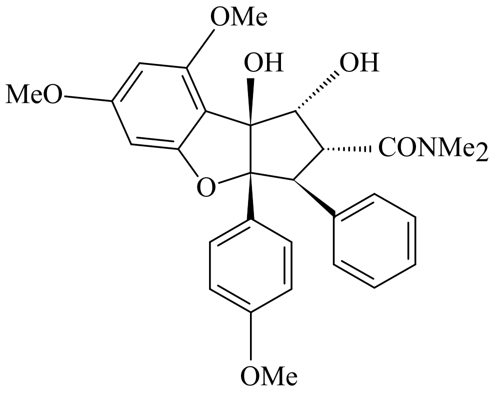
5 Meliatoxin A$_1$



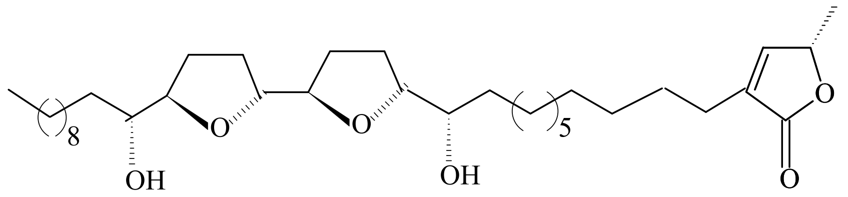
6 Meliartenin



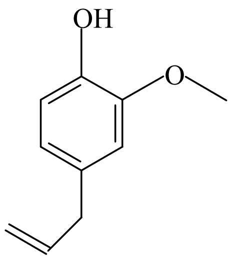
7 Toosendanin



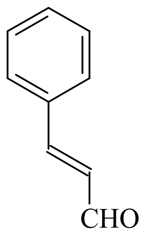
11 Rocaglamide



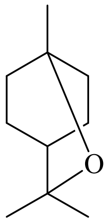
12 Squamocin-I



13 Eugenol



14 Cinnamaldehyde



15 1,8-Cineole

Fig. 6.1: Chemical structures of natural insecticides in tropical plants.

One form of *M. azedarach* native to China, is recognized as a distinct species (*M. toosendan* Sieb. & Zucc.) by botanists in that country. A wide range of bioactive limonoids have been isolated from the seeds of this tree, including salannin, meliacarpins, and an analogue of volkensin.[15] The most notable constituent however is toosendanin (**7**), which occurs in the bark at concentrations as high as 0.5%. A semi-refined bark extract containing toosendanin has been commercialized in China as a botanical insecticide. The extract contains a series of analogues of toosendanin, all of which have antifeedant effects against insects.[5]

Azadirachta excelsa

Ermel et al.[16] first reported the insect growth regulatory activity of an extract from the seeds of *A. excelsa* (Jack), as well as the isolation of a novel limonoid they called marrangin (**8**), an analogue of azadirachtin. Other investigators have isolated additional limonoids from the seeds or fruits of this lowland rainforest species, but azadirachtin itself appears to be absent. Random screening of sawdust from Malaysian timber species led to the discovery of potent insecticidal activity from one species. Bioassay driven fractionation of the wood extract led to the isolation of azadirachtin B (**2**) and three other azadirachtin analogues, two of which were novel (**9**,**10**) (Fig. 6.1).[17] The wood was later determined to be from *A. excelsa*. Given that crude extracts of *A. excelsa* stemwood are comparable in efficacy to that of neem seed extracts, and *A. excelsa* has recently been extensively planted in Malaysia as a teak substitute, a patent has been issued on the use of wood extracts for insect control, and commercialization of a botanical insecticide based on this species appears imminent.

Trichilia americana

Earlier systematic screening of the Meliaceae revealed significant bioactivity in several species in the genus *Trichilia*.[1,18-19] Among these, the Costa Rican species *T. americana* (Sesse & Mocino) Pennington proved particularly inhibitory to the Asian armyworm *Spodoptera litura*.[19] Methanolic extracts of twigs of this species dramatically prolonged larval and pupal development and decreased pupal and adult weights at dietary concentrations of 10-75 ppm fresh weight. More detailed investigation of the bioactivity of the extract indicated that the effect was a consequence of feeding inhibition alone: neither topical administration nor abdominal injection of the extract resulted in any toxicity, and when armyworms were removed from diet with the extract and allowed to feed on untainted diet, normal growth ensued.[20] In a laboratory experiment, cabbage plants sprayed with a 0.5% methanolic solution of the twig extract were almost completely protected from herbivory by 4th instar armyworms for 24 hours (Fig. 6.2).[21]

Bioassay-driven fractionation of the extract was performed in an attempt to isolate the active principle(s) therein, but was unsuccessful. The most active fraction following preparative HPLC remained chemically complex, and the putative active constituents appeared difficult to resolve and present in very small quantities. Based on phytochemical investigations of *Trichilia* species by others, it is likely that the active principles are limonoids related to hirtin.[18] The genus, which contains more than 200 species with its center of diversity in Brazil, retains considerable scope for further phytochemical exploration and biological evaluation.

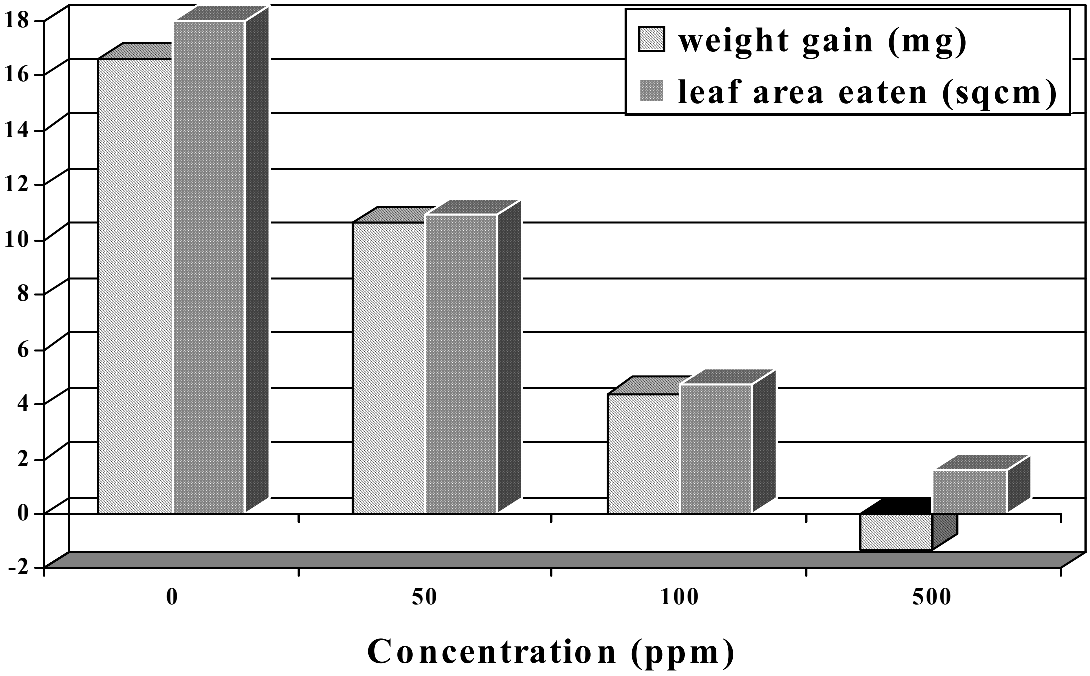


Fig. 6.2: Larval weight gain and leaf area eaten by 4th instar *Spodoptera litura* on cabbage plants sprayed with a crude twig extract from *Trichilia americana*. Adapted from reference 21.

Aglaia species

Interest in this large tropical genus was stimulated by the discovery of potent insecticidal activity in the frequently cultivated Asian species *A. odorata* Lour. Screening and phytochemical studies indicate that numerous other species in the genus are biologically active.[22-24] Bioactivity to insects can be attributed to a series of highly modified benzofurans related to rocaglamide (**11**) (Fig. 6.1); four such compounds were originally isolated from *A. odorata*.[24] In the decade since then, numerous compounds of the rocaglamide-type have been isolated from additional species of *Aglaia*, most of which inhibit insect growth or deter feeding.[25-26] Excepting azadirachtin, rocaglamide is the most potent natural insecticide isolated from higher plants to date. The mode of action of rocaglamide is inhibition of protein synthesis,[27] which accounts for its potent but slow action against insects.[28]

SPECIES OF ANNONACEAE AS SOURCES OF NATURAL INSECTICIDES

The Annonaceae, or custard apple family, is a large family of predominantly tropical trees and shrubs consisting of more than 2300 species. While certain species have enjoyed traditional use as vermifuges and for repelling lice, the broad spectrum insecticidal properties of twig extracts of the North American paw paw tree (*Asimina triloba* Dunal.) and seed extracts of the tropical sweetsop (*Annona squamosa* L.) and soursop (*A. muricata* L.) were only well documented in the past twenty years.[29-30] McLaughlin and colleagues in particular have isolated more than 100 acetogenins (*e.g.* **12**) (Fig. 6.1) – C-32 or C-34 linear fatty acids containing a 2-propanol unit to form a γ-lactone. These substances, found exclusively in the Annonaceae, are not only the insecticidal principles, but also potent anti-tumor agents. While McLaughlin has demonstrated both the efficacy of standardized extracts against pest insects and their relative safety to mammals, regulatory costs have prevented their commercialization to date.[30] Acetogenins are mitochondrial poisons, inhibiting cellular energy production through a mode of action identical to that of the well known botanical insecticide and fish poison, rotenone.[31]

Another approach to the utilization of these natural substances is the preparation of crude extracts of sweetsop and soursop seeds in developing countries for local use as crop protectants. For example, these species are widely cultivated in eastern Indonesia for the edible fruit and fruit juices they provide; in this case, the seeds are simply waste products that could be collected at minimal cost.

Annona squamosa

To determine the feasibility of the above-noted approach, seeds from soursop and sweetsop trees were collected in and around Ambon, Indonesia from 1996 to 1999. Collections of seeds from different locations and in different years were individually extracted in methanol, and their larval growth inhibitory properties were evaluated against the Asian armyworm *Spodoptera litura*. We observed around 8-fold variation in bioactivity among samples of sweetsop (*A. squamosa*) and around 6-fold variation among samples of soursop (*A. muricata*). However, sweetsop seed extracts were, on average, about 20 times more potent than those from soursop seeds.[32] Both geographic and temporal variations were significant (Fig. 6.3).

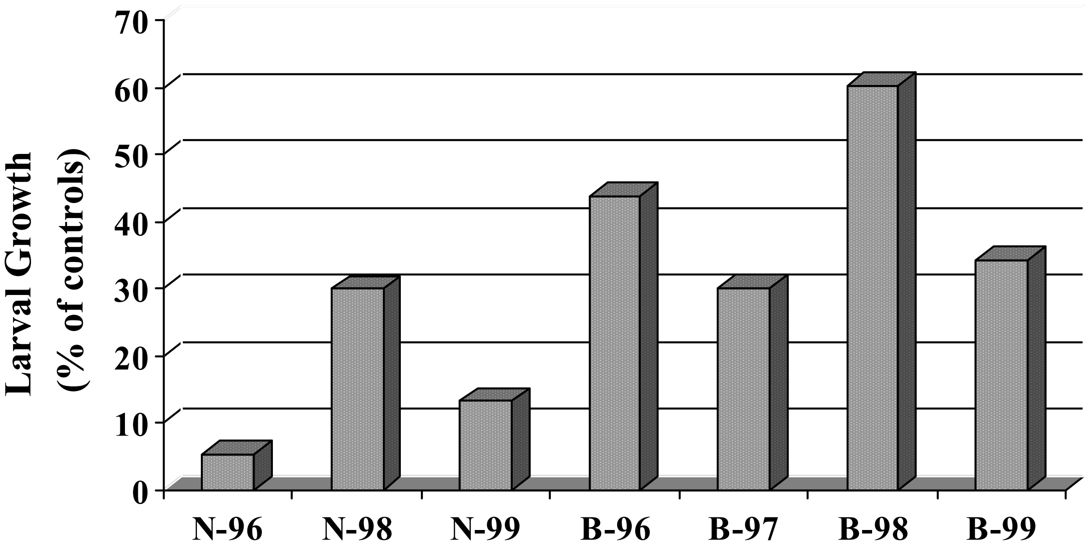


Fig. 6.3: Growth inhibitory effect of crude seed extracts of sweetsop (*Annona squamosa*) from different locations in Indonesian and years of collection on neonate *Spodoptera litura*. N = Namlea (1996-1999); B = Batugantung (1996-1999). All extracts tested at a dietary concentration of 250 ppm fwt. Adapted from reference 32.

Comparisons of the bioactivity of aqueous emulsions of methanolic seed extracts of *A. squamosa* with direct aqueous seed extracts indicated that the former are approximately 20 times more potent to 1st instar cabbage loopers (*Trichoplusia ni*) and 10 times more potent to 1st instar diamondback moth larvae (*Plutella xylostella*). Moreover, the latter insect is 13-25 times more susceptible than the former.[33]

Based on these results, we tested the efficacy of aqueous emulsions and direct aqueous extracts against diamondback moth larvae on cabbage plants in a greenhouse trial. Aqueous emulsions of seed extract as low as 0.5% concentration produced >80% larval mortality, and were superior to the use of 1% rotenone dust, a commercial botanical insecticide. An aqueous extract at 7.5% concentration produced >90% larval mortality, comparable to that obtained with 0.1% pyrethrum, another commercial botanical product.[34] Overall, these results suggest that crude local preparations based on sweetsop seeds could be effective as low cost crop protectants where the seeds are readily available.

CLOVE (MYRTACEAE) AND OTHER ESSENTIAL OIL-BEARING PLANTS AS SOURCES OF NATURAL INSECTICIDES

The most recent group of botanical products that have seen some commercial success as insecticides are the plant essential oils. Though some of these have traditional uses dating back decades, if not longer, commercialization has only taken place in the past 7-8 years. Plants producing essential oils that have been exploited for insect control include a number of herbs, most notably from the mint family (Lamiaceae), such as garden thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis*), and various species of mint (*Mentha* spp.).[35-36]

Other important sources are tropical trees, notably clove (*Syzygium aromaticum* [L.] Merr. et Perry, Myrtaceae) and cinnamon (*Cinnamomum zealanicum* Blume, Lauraceae). The phenyl propene, eugenol (**13**), is the major constituent of essential oils obtained from both clove buds (90+%) and clove leaves (45-60%), and is the active ingredient in a number of home and garden insecticides. It is particularly useful in this application owing to its rapid knockdown action on flies and crawling insects, including cockroaches, but is toxic to a range of agricultural pests as well.[37] Interestingly, eugenol is also widely used as an anaesthetic in fish research, and at high rates, as a broad spectrum natural herbicide. Essential oils prepared from cinnamon bark are rich in cinnamaldehyde (**14**), a substance with a number of useful biological actions. Products based on cinnamon oil have proven useful in the control of greenhouse pests and the fungal plant disease powdery mildew, but phytotoxicity has been a concern in commercial greenhouses growing ornamental plants. The long-standing cultivation of cloves and cinnamon in Southeast Asia and their worldwide use as culinary spices has led to their ready availability and relatively low cost, attractive features for their development and marketing as natural pesticides. Evaluation of essential oils prepared from related species of *Syzygium* and *Cinnamomum* for their pest control properties would appear to have merit. Finally, essential oils from *Eucalyptus* species (Myrtaceae), rich in the monoterpene 1,8-cineole (**15**), may have potential for exploitation as natural insecticides based on recent reports.[38] Evidence for this comes from the fact that the essential oil of rosemary (*Rosmarinus officinalis*, Lamiaceae) is the active ingredient in two botanical insecticides currently used in the United States (Hexacide^{TM} and Ecotrol^{TM}), and 1,8-cineole typically makes up approximately 50% of rosemary oil by weight (Fig. 6.1).

COMPARATIVE BIOACTIVITY OF SELECTED EXTRACTS AGAINST NOCTUID CATERPILLARS

Although all of the aforementioned plants have been demonstrated to produce extracts or oils with pronounced bioactivity against insects, few laboratories use the same insect species or employ the same bioassay protocols making direct comparisons of bioactivity impossible. Thus, my colleagues and I undertook a series of experiments in my laboratory to directly compare the efficacy of a number of botanical preparations against two important agricultural pests, the cabbage looper *Trichoplusia ni* and the true armyworm *Pseudaletia unipuncta*. To evaluate toxicity and growth inhibitory activities, neonate larvae of each species (n = 20) were placed on leaf discs (1.77 cm^2; cabbage for the looper, canary grass for the armyworm) treated with 10 µl (5 µl each side) of an aqueous emulsion of each botanical at concentrations of 0.25, 0.5, 1, or 2%. The botanicals tested in the experiments are listed in Table 61. Larvae were allowed to feed on the discs for 3 days, after which those surviving were placed individually on artificial diet and allowed to feed for a further 4 days. Larvae were weighed after the 7th day as a measure of larval growth. To measure feeding deterrence directly, 3rd instar larvae of both species were offered leaf discs treated with the test botanicals in a leaf disc choice test described elsewhere.[10]

Table 6.1: Botanical extracts tested for toxicity, larval growth inhibition and feeding deterrence in the cabbage looper and true armyworm.

Plant	Type of Extract	Active ingredient(s)
Annona squamosa (sweetsop)	Crude MeOH extract of seeds	Acetogenins (eg. squamocin-I); content unknown
Azadirachta indica (neem)	Refined extract of seeds	Azadirachtin (31%), azadirachtin B (6%)
Azadirachta excelsa (sentang)	Crude MeOH extract of stemwood	Azadirachtin analogues; content unknown
Melia toosendan (syn. *M. azedarach*)	Crude extract of bark	Toosendanin (3%) and limonoid analogues
Melia volkensii	Semi-refined extract of seeds	Mixture of limonoids; content unknown
Ryania speciosa	Dust containing powdered stemwood (50%)	Ryanodine (0.05%) and related alkaloids
Syzygium aromaticum (cloves)	Essential oil of leaves	60% eugenol
Trichilia americana	Crude MeOH extract of twigs	Unknown (limonoids?)

Extracts from both *Azadirachta* species and from *Melia volkensii* strongly inhibit early larval growth in both noctuid species, although the armyworm (*Pseudaletia*) is somewhat less susceptible (Table 6.2). In terms of insecticidal action, neem is most effective against the looper (*Trichoplusia*), whereas *M. volkensii* is most effective against the armworm. Extracts of *Ryania speciosa* wood were less active than the meliaceous species, but the level of active principle (ryanodine) is known to be low in this material (Table 6.1). The bark extract of *M. toosendan* was the least active in our bioassays, although this was expected in that the level of toosendan is rather low (3%), and this compound in purity is significantly less active than the azadirachtins found in neem (*A. indica*) and sentang (*A. excelsa*).

With respect to feeding deterrence in older larvae, the *M. volkensii* extract proved to be the most potent against the looper, whereas the neem extract was the most potent against the armyworm (Table 63). The sentang extract was somewhat less active against both species, but all of these were substantially more active than the other three extracts. *M. toosendan* and clove leaf oil (*Syzygium*) equally deterred both noctuid species, and were somewhat comparable in activity. *Ryania* was relatively ineffective as a feeding deterrent.

Table 6.2: Toxicity and growth inhibition by selected plant extracts in two species of noctuid caterpillars.

	Trichoplusia ni		*Pseudaletia unipuncta*	
	LC_{50} (%)*	EC_{50} (%)**	LC_{50} (%)*	EC_{50} (%)**
Annona squamosa	<< 0.10	<< 0.10	--	--
Azadirachta indica	<< 0.10	<< 0.10	0.83	<< 0.10
Melia volkensii	0.15	<< 0.10	0.61	0.24
Azadirachta excelsa	0.21	<< 0.10	>> 2.0	0.40
Trichilia americana	0.12	0.52	--	--
Ryania speciosa	0.35	0.65	0.88	>> 2.0
Melia toosendan	>> 2.0	--	~ 3.0	~ 2.3

*concentration causing 50% mortality, and **concentration causing 50% growth inhibition; both shown as % aqueous concentrations of extracts applied to leaf discs fed to neonate caterpillars for three days; larval weight and survival determined after seven days altogether

Table 6.3: Feeding deterrence by selected plant extracts in two species of noctuid caterpillars.

	Trichoplusia ni DC_{50} (μg cm^{-2})*	*Pseudaletia unipuncta* DC_{50} (μg cm^{-2})*
Azadirachta indica	21.9	0.6
Melia volkensii	5.8	10.8
Azadirachta excelsa	36.7	46.9
Ryania speciosa	725	400
Melia toosendan	288	249
Syzygium aromaticum	217	206

* concentration causing 50% feeding deterrence

SUMMARY

Numerous investigations worldwide have demonstrated that certain species of tropical trees produce phytochemicals with potent bioactivities against insects. A mere handful of these species have been exploited in the commercial development of botanical insecticides for crop protection, largely because bioactivity to target pests is only one of many criteria that must be satisfied for commercial development to be feasible. Other equally (if not more) important criteria are the availability of the needed starting biomass on a sustainable basis, modest cost of extraction and refinement, and stability of the active principles upon storage of the extract. Stringent regulatory requirements and the costs associated with meeting those requirements preclude access of many botanicals to the most lucrative markets in Europe and North America.

Among the botanicals discussed here, only neem has seen commercial success in North America, and that success has been limited. Yet the same factors that limit the introduction and success of botanical insecticides in highly industrialized countries should enhance their adoption in the developing countries where many of the source species grow. Many of the species discussed here are more suitable for local development and use in tropical countries where issues of standardization and stability are less critical than those of absolute cost. *Annona squamosa* is a good example of this; in rural Indonesia the starting material (seeds) is widely available at little cost, and a crude extract can be effective as a crop protectant. The same is probably true for *Melia volkensii* in its native East Africa.

At the other end of the availability spectrum, plant essential oils (viz. clove, cinnamon, eucalyptus) are currently exploited for other uses (in aromatherapy and as herbal remedies, or as flavorings) and, therefore, the infrastructure for their production is already in place, reducing their prices on the global market. Use of plant essential oils as pesticides at this point is in its infancy.

That so few botanical insecticides discovered or developed in the past 25 years have become established in the marketplace should not lessen our quest for additional botanical sources of phytochemicals with potential use in crop protection. Screening studies repeatedly confirm that the probability of finding additional species with useful bioactivity is high. We are surely more limited by scientific resources needed to characterize active principles and demonstrate their bioactivities than by the actual numbers of species in tropical forests suitable for use that remain to be investigated. It is my sincere hope that we have time to study these species before they are lost forever.

ACKNOWLEDGMENTS

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REFERENCES

1. ARNASON, J.T., MACKINNON, S., DURST, A., PHILOGENE, B.J.R., HASBUN, C., SANCHEZ, P., POVEDA, L., SAN ROMAN, L., ISMAN, M.B., SATASOOK, C., TOWERS, G.H.N., WIRIYACHITRA, P., MCLAUGHLIN, J.L., Insecticides in tropical plants with non-neurotoxic modes of action, *in*: Phytochemical Potential of Tropical Plants (K.R. Downum, J.T. Romeo, and J.A. Stafford, eds.), Plenum Press, New York. 1993, pp. 107-131.
2. MACKINNON, S., CHAURET, D., WANG, M., MATA, R., PEREDA-MIRANDA, R., JIMINEZ, A., BERNARD, C.B., KRISHNAMURTY, H.G., POVEDA, J., SANCHEZ-VINDAS, P.E., ARNASON, J.T., DURST, J., Botancials from the Piperaceae and Meliaceae of the American neotropics: phytochemistry, *in*: Phytochemicals for Pest Control (P.A. Hedin, R.M. Hollingworth, E.P. Masler, J. Miyamoto, and D.G. Thompson, eds.), American Chemical Society, Washington. 1997, pp. 49-57.
3. ISMAN, M.B., GUNNING, P.J., SPOLLEN, K.M., Tropical timber species as sources of botanical insecticides, *in*: Phytochemicals for Pest Control (P.A. Hedin, R.M. Hollingworth, E.P. Masler, J. Miyamoto, and D.G. Thompson, eds.), American Chemical Society, Washington. 1997, pp. 27-37.

4. ISMAN, M.B., Leads and prospects for the development of new botanical insecticides, *in:* Reviews in Pesticide Toxicology, Volume 3 (R.M. Roe and R. J. Kuhr, eds.) Toxicology Communications Inc., Raleigh, 1995, pp. 1-20.
5. ISMAN, M.B., MATSUURA, H., MACKINNON, S., DURST, T., TOWERS, G.H.N., ARNASON, J.T., Phytochemistry of the Meliaceae: So many terpenoids, so few insecticides, *in*: Phytochemical Diversity and Redundancy in Ecological Interactions (J. T. Romeo, J. Saunders, and P. Barbosa, eds.), Plenum Press, New York. 1996, pp. 155-178.
6. SCHMUTTERER, H. (ed.), The Neem Tree, 2nd Edition. Neem Foundation, 2002, 893 p.
7. KOUL, O., WAHAB, S. (eds.), Neem: Today and in the New Millennium. Kluwer, Dordrecht, 2004, 276 p.
8. MWANGI, R.W., Locust antifeedant activity in fruits of *Melia volkensii*, *Entomol. Exp. Appl.*, 1982, **32,** 277-280.
9. REMBOLD, H., MWANGI, R.W., *Melia volkensii* Gurke, *in*: The Neem Tree, 2nd Edition (H. Schmutterer, ed.), Neem Foundation, Mumbai. 2002, pp. 827-832.
10. AKHTAR, Y., ISMAN, M.B., Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species, *J. Appl. Ent.*, 2004, **128,** 32-38.
11. AKHTAR, Y., ISMAN, M.B., Generalization of a habituated feeding deterrent response to unrelated antifeedants following prolonged exposure in a generalist herbivore, *Trichoplusia ni, J. Chem. Ecol.*, 2004, **30,** 1333-1346.
12. ASCHER, K.R.S, SCHMUTTERER, H., MAZOR, M., ZEBITZ, C.P.W., NAQVI, S.N.H.., The Persian lilac or chinaberry tree: *Melia azedarach* L., *in*: The Neem Tree, 2nd Edition (H. Schmutterer, ed.), Neem Foundation, Mumbai. 2002, pp. 770-820.
13. KRAUS, W., Azadirachtin and other triterpenoids, *in*: The Neem Tree, 2nd Edition (H. Schmutterer, ed.), Neem Foundation, Mumbai. 2002, pp. 39-111.
14. CARPINELLA, M.C., DEFAGO, M.T., VALLADARES, G., PALACIOS, S.M., Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management, *J. Agric. Food Chem.,* 2003, **51,** 369-374.
15. XU, H.-H., CHIU, S.-F., *Melia toosendan* Sieb. & Zucc., *in*: The Neem Tree, 2nd Edition (H. Schmutterer, ed.), Neem Foundation, Mumbai. 2002, pp. 821-826.
16. ERMEL, K., KALINOWSKI, H.-O., SCHMUTTERER, H., Isolierung und charakterisierung von marrangin, einer neuen insektenmetamorphose-storenden substanz aus samenkornern des marrango-baumes *Azadirachta excelsa* (Jack), *J. Appl. Ent.*, 1991, **112,** 512-519.
17. SCHMUTTERER, H.,ERMEL, K., ISMAN, M.B., The tiam, sentang or marrango tree: *Azadirachta excelsa* (Jack), *in*: The Neem Tree, 2nd Edition (H. Schmutterer, ed.), Neem Foundation, Mumbai. 2002, pp. 760-769.
18. XIE, Y.S., ISMAN, M.B., GUNNING, P., MACKINNON, S., ARNASON, J.T., TAYLOR, D.R., SANCHEZ, P., HASBUN, C., TOWERS, G.H.N., Biological activity of extracts of *Trichilia* species and the limonoid hirtin against lepidopteran larvae, *Biochem. Syst. Ecol.*, 1994, **22,** 129-136.

19. WHEELER, D.A., ISMAN, M.B., SANCHEZ-VINDAS, P., ARNASON, J.T., Screening of Costa Rican *Trichilia* species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae), *Biochem. Syst. Ecol.*, 2001, **29,** 347-358.
20. WHEELER, D.A., ISMAN, M.B., Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*, *Entomol. Exp. Appl.*, 2001, **98,** 9-16.
21. WHEELER, D.A., ISMAN, M.B., Effect of *Trichilia americana* extract on feeding behavior of Asian armyworm, *Spodoptera litura, J. Chem. Ecol.*, 2000, **26,** 2791-2800.
22. JANPRASERT, J., SATASOOK, C., SUKUMALANAND, P., CHAMPAGNE, D.E., ISMAN, M.B., WIRIYACHITRA, P., TOWERS, G.H.N., Rocaglamide, a natural benzofuran insecticide from *Aglaia odorata, Phytochemistry*, 1993, **32,** 67-69.
23. SATASOOK, C., ISMAN, M.B., ISHIBASHI, F., MEDBURY, S., WIRIYACHITRA, P., TOWERS, G.H.N., Insecticidal bioactivity of crude extracts of *Aglaia* species, *Biochem. Syst. Ecol.*, 1994, **22,** 121-127.
24. ISHIBASHI, F., SATASOOK, C., ISMAN, M.B., TOWERS, G.H.N., Insecticidal 1H-cyclopentatetrahydro[*b*]benzofurans from *Aglaia odorata, Phytochemistry*, 1993, **32,** 307-10.
25. SCHNEIDER, C., BOHNENSTENGEL, F.I., NUBROHO, B.W., WRAY, V., WITTE, L., HUNG, P.D., KIET, L.C., PROKSCH, P., Insecticidal rocaglamide derivatives from *Aglaia spectabilis* (Meliaceae), *Phytochemistry*, 2000, **54,** 731-736.
26. GREGER, H., PACHER, T., BREM, B., BACHER, M., HOFER, O., Insecticidal flavaglines and other compounds from Fijian *Aglaia* species, *Phytochemistry*, 2001, *57,* 57-64.
27. OHSE, T., OHBA, S., YAMAMOTO, T., KOYANO, T., UMEZAWA, K., Cyclopentabenzofuran lignan protein synthesis inhibitors from *Aglaia odorata, J. Nat. Prod.*, 1996, **59,** 650-652.
28. SATASOOK, C., ISMAN, M.B., WIRIYACHITRA, P., Toxicity of rocaglamide, an insecticidal natural product to the variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae), *Pestic. Sci.*, 1993, **36,** 53-58.
29. RUPPRECHT, J.K., HUI, Y.-H., MCLAUGHLIN, J.L., Annonaceous acetogenins: A review, *J. Nat. Prod.*, 1990, **53,** 237-278.
30. MCLAUGHLIN, J.L., ZENG, L., OBERLIES, N.H., ALFONSO, D., JOHNSON, J.A., CUMMINGS, B.A., Annonaceous acetogenins as new natural pesticides: recent progress, *in*: Phytochemicals for Pest Control (P.A. Hedin, R.M. Hollingworth, E.P. Masler, J. Miyamoto, and D.G. Thompson, eds.), American Chemical Society, Washington. 1997, pp. 117-133.
31. LONDERSHAUSEN, M., LEICHT, W., LIEB, F., MOESSCHLER, H., Molecular mode of action of annonins, *Pestic. Sci.*, 1991, **33,** 427-433.
32. LEATEMIA, J.A., ISMAN, M.B., Insecticidal activity of crude seed extracts of *Annona* spp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae, *Phytoparasitica*, 2004, **32,** 30-37.
33. LEATEMIA, J.A., ISMAN, M.B., Toxicity and antifeedant activity of crude seed extracts of *Annona squamosa* (Annonaceae) against lepidopteran pests and natural enemies, *Intl. J. Tropical Insect Sci.*, 2004, **24,** 1-10.

34. LEATEMIA, J.A., ISMAN, M.B., Efficacy of crude seed extracts of *Annona squamosa* against diamondback moth, *Plutella xylostella* L. in the greenhouse, *Intl. J. Pest Mgmt.*, 2004, **50,** 129-133.
35. ISMAN, M.B., Pesticides based on plant essential oils, *Pestic. Outlook*, 1999, **10,** 68-72.
36. ISMAN, M.B., Plant essential oils as green pesticides for pest and disease management, *in*: Agricultural Applications in Green Chemistry (W.M. Nelson, ed.), American Chemical Society, Washington. 2004, in press.
37. ISMAN, M.B., Plant essential oils for pest and disease management, *Crop Protection*, 2000, **19,** 603-608.
38. CHOI, W.-I., LEE, S.-G., PARK, H.-M., AHN, Y.-J., Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae), *J. Econ. Entomol.*, 2004, **97,** 553-558.