Pesticide Research Journal Vol 6(1) 11-19., June 1994

Botanical Insecticides and Antifeedants : New Sources and Perspectives

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Although hundreds of plant natural products (allelochemicals) have demonstrated deleterious effects on insects in laboratory bioassays, only a handful of botanical insecticides are currently approved for use in industrialized countries. Two main factors may account for this dichotomy. Firstly, insecticide discovery often relies on bioassays which focus on acute toxicity; as a consequence, important sublethal or chronic effects may be completely overlooked. Secondly, commercialization of botanical preparations for insect control may hinge more on practical concerns (e.g. availability of large quantities, management of chemical variability) than on biological ones (e.g. efficacy, favorable non-target toxicity). Antifeedants continue to attract considerable interest in the research community, but their utility for insect control remains questionable. Potential problems with antifeedants as crop protectants (e.g. interspecific variation) are discussed. However, recent studies suggest that many allelochemicals described as antifeedants in the literature may have a broader range of physiological effects on insects. A more comprehensive program of bioassays used in the screening of plant extracts or isolated compounds may help uncover new plant material that have the potential to be developed into botanical insecticides.

KEY WORDS : Botanical insecticides, antifeedants, insect growth regulators

While advances in biotechnology hold promise for the genetic engineering of plants, insects and microorganisms with the ultimate goal of crop protection, most experts agree that it will be at least two decades before these technologies completely displace pesticides. Until then, pesticides will remain the cornerstone of integrated pest management, althought changing consumer demands and the increasing attention to environmental protection and preservation may render many long-standing pesticides no longer acceptable. Pest control products generated in the 1990's will require improved safety and environmental profiles, in terms of safety to both the user and consumer of treated crops[1]. These products also need to be compatible with biological control agents to ensure their place in integrated pest management.

Botanical insecticides could to a large extent meet the demands for safe and environmentally-sound crop protectants. Existing and previously used commercial botanical pesticides vary widely in their vertebrate toxicity, and we cannot assume safety until demonstrated conclusively by laboratory testing on animals. However, some botanicals show vertebrate selectivity comparable to the safest synthetic products. On the other hand, all botanicals can be characterized as environmentally non-persistant, and therefore are unlikely to result in environmental damage. Also, most botanicals are described as "stomach poisons" indicating that they lack contact toxicity but instead must be ingested by the target pest to be effective. The advantage therein is that natural enemies (which are not phytophagous), will not acquire a toxic dose by merely contacting foliar residues, as can readily occur with potent synthetic insecticides such as the modern pyrethroids.

At present, only two botanical insecticides enjoy worldwide acceptance and use:

pyrethrum and rotenone. Pyrethrum, though containing neurotoxic esters with good contact action, is relatively safe to vertebrates and is rapidly broken down in the environment. Rotenone, in contrast, is relatively toxic to vertebrates (rat toxicity is comparable to that of DDT), and although it too is degraded in the environment, its' extreme toxicity to fish limits its' use near waterways. Numerous other traditional plant preparations are in local use (e.g. ryania, sabadilla, quassia),[2,3] but none of these are likely to be further developed for widespread use in industrialized countries.

Neem *(Azadirachta indica)* represents the leading edge of a new wave of botanical insect control products[4]. It satisfies almost all of the desirable criteria for a pesticide: broad spectrum of action against pests, minimum disruption of natural enemies, no appreciable vertebrate toxicity or phytotoxicity, rapid environmental breakdown, and available from a regionally abundant, renewable resource. The active principle of neem, azadirachtin, has probably been subjected to more studies in insects than any other plant natural product to date[5-7]. Certainly no other natural insecticide has been the focus for as many international conferences and symposia as has neem. In spite of this acknowledged acceptance in the research community, commercial acceptance of neem, at least in North America and Europe, is still in its' infancy. Two U.S. companies (W.R. Grace, Agridyne Technologies) have neem-based insecticides approved by the Environmental Protection Agency; one of these products (Align™, Agridyne) is approved for use on food crops. A Canadian company (PheroTech) has a neem insecticide which will enter the registration process in 1994. In spite of the years of neem research in England and Germany, there are no neem products under development for use in western Europe. Nonetheless, commercial success of neem, at least in North America, will to a large extent dictate the future for other botanicals to follow.

Acute toxins, growth regulators and growth inhibitors

Plant natural products isolated and identified to date number in the thousands, and increasingly, newly discovered natural products are being routinely subjected to bioassay in the search for new pharmaceuticals. To a lesser, but no less significant extent, these compounds are finding their way into the hands of entomologists with the result that we now know of hundreds of plant chemicals that are deleterious or at least behaviorally deterrent to one or more species of insect. As one ecologist recently stated, "Demonstrating that plant secondary compounds are toxic, repellent, or growth-inhibiting to herbivores has become a minor industry supporting professional ecologists"[8]. Why then are there so few botanical insecticides in widespread use or in the final stages of commercialization?

I believe that one of the reasons behind this traditional dichotomy between science and practice has been the industry focus on acute insecticidal action. Because most conventional chemical screening is based on bioassays of acute toxicity (with mortality most often observed at 24 or 48 hours[9]), we have tended to select substances which are the most potent and rapid in action. It should not be surprising then, that all botanical insecticides in current use, save neem, are neurotoxins or muscle poisons. Unfortunately, because of similarities in vertebrate and insect neuromuscular physiology, toxins relying on these modes-of-action tend to be inherently toxic to warm-blooded animals, and selectivity must depend on differences in pharmacokinetics and detoxicative metabolism.

In contrast, chemically-mediated insect-plant interactions in nature are far more subtle. Most plant defensive chemicals (*i.e.* allelochemicals) discourage insect herbivory, either by deterring feeding and oviposition or by inhibiting larval growth, rather than by killing insects outright. Thus, the search for botanical insecticides with bioactivity comparable to that of synthetic neurotoxic insecticides is a search for biological exceptions. In my laboratory, one standard bioassay is based on the growth of neonate noctuid larvae *(e.g. Peridroma saucia, Spodoptera litura)* on artificial diets in which plant extracts or pure compounds are incorporated. By this means, we have documented larval growth inhibition by a wide range of plant natural products and plant preparations, yet none of these materials have rapid contact toxicity of the type seen with application of pyrethrum. At proportionally higher concentrations, substances which are profoundly inhibitory to growth result in larval mortality, but this is likely a consequence of starvation (from absolute feeding deterrence) rather than toxicity *per se.* Usually this effect is only seen at concentrations 5-10 times higher than that resulting in 50% inhibition of larval growth, relative to control larvae (i.e. the dietary EC_{50} value is 5-10 times lower than the dietary LC_{50}.

One can envision how a potent growth inhibitor could act as a crop protectant: prolongation of the larval stage of a pest insect will accentuate the natural hazards to which the pest will be exposed, both biotic and abiotic. Life table studies with noctuids indicate that 80% of larval mortality occurs before the third instar, and cage exclusion experiments confirm that natural enemies (parasitoids and predators) account for much of this mortality. Furthermore, pest larvae impaired by a growth inhibitor may be forced to spend more of their time searching for acceptable food, thus increasing their vulnerability to both natural enemies and climatic factors. Finally, as mortality would result from several causes, selection for resistance to the growth inhibitor would be diffused.

Even in cases where a single dose of a plant toxin results in significant mortality, a 24 or 48 hour observation period may be insufficient to observe the full extent of toxicity. Two recent examples from my laboratory illustrate this point. Azadirachtin, administered topically to fifth instar nymphs of the migratory grasshopper *(Melanoplus sanguinipes)* at 5 µg/g, causes no discernable effects through the 7-day course of the instar, but more than half the treated nymphs ultimately die in a failed molt to the adult stage[10]. We recently isolated the highly-subtituted benzofuran, rocaglamide, as the insecticidal principle of the ornamental shrub *Aglaia odorata* (Meliaceae)[11]. This compound, following topical administration, has an LD_{50} in fourth instar variegated cutworms *(Peridroma saucia)* of 0.32 µg/larva[12]. However, all treated larvae remain alive at 24 hours; mortality does not become apparent until at least 72 hours, and is not fully manifested until 4-5 days. Thus, bioassay of these two compounds based on a 24 or 48 hour assessment of mortality would have completely overlooked their profound bioactivity.

Antifeedants

The concept of using non-toxic feeding deterrents (antifeedants) has attracted considerable attention ever since the striking antifeedant effects of azadirachtin was first documented in the desert locust *(Schistocerca gregraria)*[13]. Antifeedants have been the subject of several recent reviews[14,15], but there have been very few attempts to demonstrate their potential utility for crop protection[16].

Some authors apply a very liberal definition for an antifeedant, namely, any substance which reduces consumption by an insect. However, I propose a more restrictive definition: a peripherally-mediated behavior-modifying substance (i.e. acting directly on the chemosensilla) resulting in feeding deterrence. This latter definition thus excludes chemicals which might suppress feeding through centrally-mediated effects. For example, *topical* application of rocaglamide to cutworms results in marked feeding suppression[12]. Clearly, this is an anorexic effect, which could be a consequence of an action on either the insects central nervous system (e.g. the feeding centre in the suboesophogeal ganglion) or on the alimentary canal. Azadirachtin produces a similar overall effect on consumption[17], as do several other allelochemicals. It has even been suggested that plants can 'psychomanipulate' insects by interfering with information processing in the central nervous system[18]. Recent studies have revealed that certain germacranolide sesquiterpene lactones isolated from cultivated sunflower as antifeedants to rootworms *(Diabratica virgifera* also produce, upon injection, neurotoxic symptoms consistent with that of a γ- aminobutyric acid ($GABA_A$)-gated chloride channel antagonist[19]. A picrotoxinin-like receptor appears to mediate both the antifeedant effects in the gustatory sensilla and the central toxic effects of these sesquiterpene lactones[20].

Unfortunately, antifeedants have a number of drawbacks as crop protectants. although azadirachtin is an outstanding exception, most antifeedants might lack any appreciable *systemic* action in plants. As a consequence, within one or two days after application, pest insects may be diverted to feeding preferentially on the growing tissues of plants, which would be unprotected. Even if plant growth is very slow, the antifeedant may lose its' ability to protect foliage, not because of inadequate persistance of the chemical on foliage, but because the pests may *habituate* to the antifeedant. Because the sensitivity of insect chemosensilla can decline markedly with exposure to a particular stimulus[21,22,] the protectant action in the field could be very short-lived.

From the industrial perspective, the greatest problem with antifeedants is the specificity, or perhaps better stated, interspecific differences among pests with respect to sensitivity. Azadirachtin again provides a case in point. Owing to its outstanding antifeedant activity against the desert locust (EC_{50} = 0.05 ppm), we expected a comparable level of activity against the migratory grasshopper, a major pest of cereal crops and rangeland in North America. To our surprise, this species was completely insensitive (EC_{50} > 1000 ppm)[10]. However, the insect growth regulatory (molt disrupting) effects of azadirachtin in the two species are comparable.[17] When azadirachtin was added to an artificial diet, there were no significant differences in the degree of growth inhibition between the species (EC_{50} = 0.12-0.24 ppm.). In contrast, there were highly significant differences in feeding deterrency. In a diet choice test employing second instar larvae, only two of the six species of noctuids were deterred from feeding on diet at a concentration that would result in severe inhibition of growth (0.40 ppm). In a leaf-disc choice test employing fourth instar larvae, EC_{50} values varied more than 30-fold, with *spodoptera litura* the most sensitive, and *Actebia fennica* the least. The salient point is that interspecific behavioral responses to allelochemicals appear far more plastic than do physiological effects.

This last point was also well demonstrated by a collaborative study in my laboratory in which we tested 14 allelochemicals for bioactivity against two species of noctuids *Peridroma saucia* and *Mamestra configurata*[23]. All of the compound tested were potent antifeedants to stored product coleopterans[24]. However, based on leaf-disc choice tests, only three of the 14 compounds were significant antifeedants for *Peridroma*. On the other hand, the same three were also significant growth inhibitors following topical application to fourth instar larvae. Toxicity of one of the compounds, the sesquiterpene bisabolangelone, was confirmed by topical application to the milkweed bug nymphs, *Oncopletus fasciatus*. These results indicate that screening and evaluation of plant natural products based on their antifeedant effects alone may easily overlook physiological effects which could prove to be even more important and potentially useful.

Although behavioral effects of plant natural products should not be overlooked, I recommend a bioassay strategy which focusses primarily on the physiological actions against insects. This strategy would include bioassays with endpoints for growth inhibition, growth disruption (IGRs), and acute toxicity. Incorporation of plant extracts, fractions there of, or isolated compounds into artificial diets is a convenient means of assessing larval growth inhibition, but because any bioassay where insects are orally exposed to test substance does not discriminate between growth inhibition mediated by behavioral deterrence (i.e. antifeedant effects) and that resulting from post-ingestive toxicity (physiological effects,) positive results need to be confirmed by a separate bioassay (e.g. topical application) where the results cannot be confounded by feeding behavior. Significant contact toxicity will also be expressed in the diet incorporation bioassay (as high mortality in the first or second day), and can then be confirmed by other bioassays using other routes of administration (e.g. topical application, or application to glass surfaces[25]). Growth regulatory activity can sometimes be missed in a diet study using small lepidopteran larvae, and for that reason, we routinely test for molt disruption using the *Tenebrio* pupal bioassay[26], or topical application to last instar *Oncopeltus* nymphs[27]. Both of these latter insects are relatively easy to maintain in the laboratory, and are very sensitive to endocrine disruption.

New sources for botanical insecticides

Tropical plants remain a vast, virtually untapped source of potentially useful phytochemicals. However, random screening of plants from a particular region is not likely the most efficient strategy for the discovery of new sources of bioactive compounds. While many plant families have not been investigated to date, previous screening efforts point to particular families where the probability of finding species with bioactivity against insects is enhanced. Of these, the mahogany family (Meliaceae), to which neem belongs, appears to be a rich source[28,29]. In addition to *Azadirachta*, a considerable number of insect growth regulators and antifeedants have been isolated from the genera *Melia* and *Toona* by Kraus and co-worker[30].

The Meliaceae consists of approximately 50 genera and 500 species[31]. As many of these genera had not been investigated previously, we conducted a systematic investigation in which we screened over 75 species representing 25 genera of Meliaceae collected by us and collaborators throughout the Indo-Malaysian region and Central America. All of our extracts were screened for growth inhibitory activity against the variegated cutworm (*Peridroma saucia*, a highly polyphagous noctuid that we consider a conservative bioassay species. In addition, all of our Central American extracts have also been screened against the European corn borer (*Ostrinia nubilalis*)[32]. Our initial screening concentration is 0.2% fresh weight of extract in artificial diet (= 2000 ppm). When bioassay-driven fractionation results in the isolation of pure constituents, these are screened at a dietary concentration of 50 ppm.

In our survey, we uncovered strong bioactivity (80% inhibition of larval growth at 0.2% fwt) in six genera, and one related genus outside of the Meliaceae. Three of these six (*Turrea, Chisocheton, Didymocheton*) had not been previously screened for bioactivity against insects. Our investigation, combined with studies by other laboratories indicate to date that at least ten families within the Meliaceae are fruitful sources of phytochemicals bioactive in insects[33]. We focussed largely on two genera, *Aglaia* and *Trichilia*. Of 19 species of *Aglaia* screened, more than a third were significantly bioactive[34], while six of 11 species of *Trichilia* were very active[35]. Given that these two genera each consist of more than one hundred species, there obviously remains considerable scope for discovery of additional insecticidal species in these genera alone.

Following several unsuccessful attempts, our colleagues in Thailand were able to isolate the active principle from *A. odorata* and identify it as the highly substituted benzofuran, rocaglamide[11], previously isolated as an antileukemic agent from the closely related *A. elliptica*. Further phytochemical investigation led to the isolation of three additional analogues, all of which are insecticidal[36]. We are currently attempting to isolate the active principles from species of *Chisocheton* and *Trichilia*.

Even though there are numerous plant species that show promising bioactivity against insects, the majority of these are unlikely to serve as the starting point for a botanical insecticide. Why? Whenever industry becomes engaged in discussions of prospective botanicals, the first question asked is: how abundant and dependable is the supply of plant material? Commercial development is not feasible unless tonne quantities of plant material can be readily sourced, immediately limiting plant species to those which are readily abundant in nature, or those which can be readily propagated and cultivated, and from which the desired tissue can be harvested in a non-destructive manner (i.e. a sustainable resource). Obviously, neem meets these criteria well, but it may represent a biological exception.

A completely different strategy involves investigating industrial byproducts from the agrifood and forest industries, where starting material is already available in quantity, often at minimal cost. For example, seeds are waste products of the fruit juice industry; at least two botanical insecticides could be developed from this resource. The isolation of insecticidal acetogenins from the seeds of soursop and sweetsop (*Annona* spp.) is well established[37], as is the isolation of antifeedant limonins from grapefruit seed[38]. The lumber industry produces enormous quantities of woodwaste and sawdust. Given that certain species of Meliaceae are harvested for timber throughout the tropics (e.g.*Cedrela, Swietenia, Entandrophragma, Khaya*), it may be possible to obtain large quantities of limonoids from this resource. Our screening of crude extracts from wood samples collected at sawmills in Malaysia and Indonesia suggests that timber species in other tropical plant families may also yield extractives which might be developed as crop protectants.

Several botanical materials with potential for commercialization as insecticides and antifeedants have been recently reviewed[14].

Summary and recommendations

Tropical plants provide fertile ground for the phytochemical prospector. In turn, newly identified phytochemicals may have striking bioactivity against insects, with novel modes-of- action. To take best advantage of this rich resource, an active partnership between the chemist and entomologist is required. The entomologist is responsible for conducting reliable, reproducible bioassays with clearly defined endpoints, to guide the phytochemist in his effort to isolate active principles from the plant under investigation. If the chemist is successful, then the entomologist must ensure that the full extent of bioactivity is determined for each substance isolated, by conducting a broad range of bioassays, including those specifically designed to measure sublethal growth inhibition or insect growth regulatory effects in addition to contact toxicity.

While it is conceivable that the discovery of a new insecticidal natural product from a rare plant could serve as the lead for synthetic chemistry, and this philosophy is widely touted in the agrochemical industry, there are hardly any examples, apart from the pyrethroids, of commercial insecticides that can trace their origins directly to a plant natural product. It may therefore be more profitable for researchers to place more emphasis on the search for bioactive compounds from plant material available in abundance, such as waste products and byproducts from the food and forestry industries.

ACKNOWLEDGMENTS

I thank Dr. Ken Naumann for critical review of the manuscript, and the Natural Sciences and Engineering Research Council (Canada) for generous support of my research program.

REFERENCES

1. Finney, JR (1990) Where do we stand-where do we go? *Proc 7th Int Conf Pestic Chem* Hamburg 1990, pp 1- 26
2. Crosby, DG (1971) Minor insecticides of plant origin. In *Naturally Occurring Insecticides* (M Jacobson and DG Crosby, Eds) pp 177-239 Marcel Dekker Inc, New York
3. Ahmed S, Grainge M, Hylin JW, Mitchell WC and Litsinger JA (1984) Some promising plant species for use as pest control agents under traditional farming systems *Proc 2nd Int* Neem *Conf* Rauischholzhausen 1983, pp 565-580
4. Isman MB (1994) Leads and prospects for the development of new botanical insecticides *Rev Pestic Toxicol* **3**: 1-20
5. Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, *Azadirachta indica Ann Rev Entomol* 35: 271-297
6. Gujar GT (1992) Neem (*Azadirachta indica* A Juss), a natural insecticide: status and need for bioassay standards *Pestic* Res J **4**: 69—79
7. Mordue (Luntz) AJ, Blackwell A (1993) Azadirachtin: an update *J Insect Physiol* **39**: 903-924
8. Rausher MD (1992) Natural selection and the evolution of plant-insect interactions. In *Insect Chemical Ecology. an evolutionary approach* (BD Roitberg & MB Isman, Eds) pp 20-88 Chapman and Hall, New York
9. Leeper JR, Raffa KF (1986) Baseline data for evaluating resistance development: dosage/ mortality studies using two test methods with chlorpyrifos, methomyl and permethrin in *Heliothis viresecens* (F). and *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) *Trop Pest Mgmt* **32**: 137-145
10. Champagne DE, Isman MB, Towers GHN (1989) Insecticides activity of phytochemicals and extracts of the Meliaceae. In *Insecticides cf Plant Origin* (JT Arnason, BJR Philogene, P Morand, Eds) pp 95-109 American Chemical Society, Washington
11. Janprasert J, Satasook C, Sukumalanand P, Champagne DE, Isman MB, Wiriyachitra P, Towers GHN (1993) Rocaglamide, a new natural insecticide from *Aglaia odorata* (Lour) (Meliaceae) *Phytochemistry* **32**: 67-70

12. Satasook C, Isman MB, Wiriyachitra P (1992) Activity of rocaglamide, an insecticidal natural product, against the variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae) *Pestic* Sci **36:** 53-58

13. Butterworth JH, Morgan ED (1968) Isolation of a substance that suppresses feeding in locusts *J Chem Soc, Chem Comm* pp 23-24

14. Frazier JL (1986) The perception of plant allelochemicals that inhibit feeding. In *Molecular Aspects of Insect-Plant Associations* (LB Brattsten, S Ahmad, Eds) pp 1-42 Plenum Press, New York

15. Jermy T (1990) Prospects of antifeedant approach to pest control- a critical review *J Chem Ecol* **16:** 3151-3166

16. Murray KD, Alford AR, Groden E, Drummond FA, Storch RH, Bentley MD, Sugathapala PM (1993) Interactive effects of an antifeedant used with *Bacillus thuringiensis var. San diego* delta endotoxin on Colorado potato beetle (Coleoptera: Chrysomelidae) *J Econ Entomol* **86:** 1793-1801

17. Isman MB (1993) Growth inhibiory and antifeedant effects of azadirachtin on six noctuids of regional economic importance *Pestic Sci* **38:** 57-63

18. Murdock LL, Brookhart G, Edegecomb RS, Long TF, Sudlow L (1985) Do plants "*Psychomanipulate*" insects? In *Bioregulators for Pest Control* (PA Hedin, Ed) pp 337-351 American Chemical Society, Washington

19. Mullin CA, Alfatafta AA, Harman JL Serino AA, Everett SL (1991) Corn rootworm feeding on sunflower and other Compositae. Influence of floral terpenoid and phenolic factors. In *Naturally Occurring Pest Bioregulators* (PA Hedin, Ed) pp 278-292 American Chemical Society, Washington

20. Mullin CA, Mason CH, Chou JC, Linderman JR (1992) Phytochemical antagonism of γ-aminobutyric acid based resistances in *Diabraotica*. In *Molecular Mechanisms of Insecticide Resistance* (CA Mullin, JG Scott, Eds) pp 288-308 American Chemical Society, Washington

21. Schoonhoven LM (1982) Biological aspects of antifeedants *Entomol Exp Appl* 31: 57-69

22. Blaney WM, Schoonhoven LM, Simmonds MSJ (1986) Sensitivity variations in insect chemoreceptors; a review *Experientia* **42:** 13-19

23. Nawrot J, Koul O, Isman MB, Harmatha K (1991) Naturally occurring antifeedants: effects on two polyphagous lepidopterans *J Appl Ent* **112:** 194-201

24. Nawrot J, Bloszyk E, Harmatha J, Novotny L (1986) Action of antifeedants of plant origin on beetles infesting stored products *Acta Ent Bohemoslov* **83**:327- 335

25. Isman MB, Proksch P, Yan JW (1987) Insecticidal chromenes from the Asteraceae: structure-activity relations *Entomol Exp Appl* **43:** 87-93

26. Bowers WS (1968) Juvenile hormone: activity of natural and synthetic synergists *Science* **161:** 895-897

27 Isman MB, Koul O, Luczynski A, Kaminski J (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin *J Agric Food Chem* **38:** 1406-1411

28. Jacobson M (1989) Botanical pesticides. Past, Present, and future. In *Insecticides of Plant Origin* (JT Arnason, BJR Philogene, P Morand,, Eds) pp 1-10 American Chemical Society, Washington

29. Schmutterer, H (1992) Higher plants as sources of novel pesticides. In *Insecticides: Mechanisms of Action and Resistance* (D Otto, B Weber, Eds) pp 3-15 Intercept, Andover

30. Kraus W, Kypke K (1979) Surenone and surenin, two novel tetranortriterpenoids from *Toona sureni* (Blume) Merrill *Tetrahedron Lett* **29:** 2715-2716

31. Pennington PD, Styles BT (1975) A generic monograph of the Meliaceae *Blumea* **22:** 419-540

32. Arnason JT, Mackinnon S, Durst A, Philogene BJR, Hasbun C, Sanchez P, Poveda L, San Roman L, Isman MB, Satasook C, Towers GHN, Wiriyachitra P, Mclaughlin JL (1993) Insecticides in tropical plants with non-neurotoxic modes of action. In *Phytochemical Potential of Tropical Plants* (KR Downum, JT Romeo, HA Stafford, Eds) pp 107-131 Plenum Press, New York

33. Isman MB, Arnason JT, Towers GHN (1994) Other species 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* (H Schmutterer, Ed) VCH, Weinheim, in press

34. Satasook C, Isman MB, Ishibashi F, Medbury S, Wiriyachitra, P, Towers GHN (1994) Insecticidal bioactivity of crude extracts of *Aglaia* species (Meliaceae) *Biochem Syst Ecol,* (in press)

35. Xie YS, Ismam MB, Gunning P, Mackinnon S, Arnason JT, Taylor DR, Sanchez P, Hasbun C, Towers GHN (1994) Biological activity of extracts of *Trichilia* species and the limonoid hirtin against lepidopteran larvae *Biochem Syst Ecol,* (in press)

36. Ishibashi F, Satasook C, Isman MB, Toweres GHN (1993) Insecticidal 1H cyclopentatertahydro [b] benzofurans from *Aglaia odorata* (Lour.) (Meliaceae) *Phytochemistry* **32:** 307-310

37. Moeschler HF, Pfluger W, Wendlisch D (1987) Pure annonin and a process for the preparation thereof. US Patent No 468923

38. Klocke JA, Kubo 1 (1982) Citrus limonoid byproducts as insect control agents *Entomol Exp Appl* 32: 299-301

Manuscript received March 5, 1994. Accepted May 30, 1994.