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Editorial

Fighting the global pest problem: preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control

Graham M. Nicholson

Neurotoxin Research Group, Department of Medical & Molecular Biosciences, University of Technology, Sydney, PO Box 123, Broadway, NSW, 2007, Australia

* Corresponding author. Associate Professor Graham Nicholson, PhD
Neurotoxin Research Group
Department of Heath Sciences
University of Technology, Sydney
PO Box 123
Broadway, NSW, 2007
Australia
Tel.: +61-2-9514-2230; fax: +61-2-9514-2228
E-mail address: Graham.Nicholson@uts.edu.au

Abstract

Arthropod pests are responsible for major crop devastation and are vectors for the transmission of new and re-emerging diseases in humans and livestock. Despite many years of effective control by conventional agrochemical insecticides, a number of factors are threatening the effectiveness and continued use of these agents. These include the development of insecticide resistance and use-cancellation or de-registration of some insecticides due to human health and environmental concerns. Several approaches are being investigated for the design of new (bio)pesticides. These include the development of transgenic plants and recombinant baculoviruses as delivery systems for a variety of insect-selective toxins. Additional approaches for the development of foliar sprays include the rational design of peptidomimetics based on the key residues of these toxins that interact with the insect target. This special issue provides an overview of these phyletically-selective animal, plant and microbial toxins and their diverse mechanisms of action to paralyze or kill arthropods. In addition, it reviews their potential for biopesticide discovery and validation of novel insecticide targets and provides an overview of the strengths and weaknesses of biopesticides in the global control of arthropod pests.

Keywords: insecticide, insect-selective toxin, biopesticide, baculovirus, peptidomimetic, transgenic plants

1. The global pest problem

1.1 Crop pests

Arthropods are undoubtedly the most widespread and diverse groups of animals, with an estimated 4-6 million species worldwide (Novotny et al., 2002). While only a small percentage of arthropods are classified as pest species, they nevertheless cause major devastation of crops, destroying around 18% of the world annual crop production (Oerke and Dehne, 2004), contributing to the loss of nearly 20% of stored food grains (Bergvinson and Garcia-Lara, 2004), and causing around US$100 billion damage each year (Carlini and Grossi-de-Sa, 2002).

Phytophagous (plant-eating) insect and mite pests are a major threat to food production for human consumption. Without the use of chemical insecticides dramatic losses in worldwide crop yield would occur. In 2001, a total of US$7.56 billion was spent in order to protect crops from damage by invertebrate phytophagous pest species (Beckmann and Haack, 2003). Larval forms of lepidopterans

are considered the most destructive insects, with about 40% of all chemical insecticides directed against heliothine species (Brooks and Hines, 1999). The Entomological Society of America indicates that over 25% of the 300 primary insect pests in the United States belong to the Order Lepidoptera. Although, many species within the Orders Acarina, Coleoptera, Diptera, Hemiptera, Orthoptera and Thysanoptera are also considered agricultural pests.

An increasing world population will place growing demands on crop production in order to avert escalating malnutrition. While the world human population is currently 6.5 billion, the U.S. Census Bureau projects a global population of over 9.2 billion by the year 2050, an increase of over 40% (U.S. Census Bureau, 2006). Due to this increasing world population, the world will have to produce more food in the next 50 years than it has since the onset of agricultural production approximately 10,000 years ago. To compound the problem, almost all of the world's fertile land is currently in use and arable land areas cannot be expanded significantly. For this reason, the global challenge is to secure high and quality yields and to make agricultural production environmentally compatible. Improvement in pest control strategies represents one method to generate higher quality and greater quantity of agricultural products.

1.2 Insects as vectors of disease

Many pests, particularly hematophagous (blood-sucking) insects, act as vectors for the transmission of an array of previously unrecognized vector-borne human and veterinary diseases. Moreover, they also act as vectors for the resurgence of diseases that have been relatively dormant or under control. Disease vectors such as mosquitoes, ticks, fleas, lice and triatomid bugs are of public health importance and are of increasing concern to the general population, particularly in third world countries (Gubler, 1998b; 2002). Therefore they pose a threat to the productivity, health, and well being of humans, livestock, companion animals and wild life (Brogdon and McAllister, 1998; Gubler, 1998b).

Mosquitoes are probably the most pernicious example of an insect vector, involved in the transmission of malaria, dengue-dengue hemorrhagic fever, yellow fever, filariasis, Japanese encephalitis, Rift Valley fever, West Nile virus and various flaviruses (Gratz, 1999; Gubler, 2002; Mackenzie et al., 2004). Presently, malaria is endemic in 100 countries or territories, although the areas where transmission takes place have reduced, and it is now confined to inter-tropical zones (Fig. 1A). However, the number of people living at risk has grown to about 3 billion, or around 46% of the world's human population, and is anticipated to continue increasing. Malaria is responsible for up to 500 million new clinical cases world wide each year causing at least 100 million cases of acute illness, with 90% in Africa, and between 1.5 and 2.7 million deaths per year, again mostly in Africa (World

Health Organisation, 1997; Breman, 2001) (Fig. 1C). In addition to diseases transmitted by mosquitoes, a variety of arthropod pests such as sand flies, tsetse flies, ticks, fleas, triatomid bugs, and lice are responsible for the transmission of many new and reemerging human diseases. These include onchocerciasis, Chagas disease, Bartonella, leishmaniasis, trypanosomiasis, plague, ehrlichiosis, typhus and various rickettsioses as well as Lyme disease, the most prevalent vector-borne disease in North America (Gubler, 1998a; Gratz, 1999; Gubler, 2002).

Arthropod-borne diseases not only affect human populations but also affect global food production. In many areas of the world, particularly the tropics, these diseases compromise efficient production of domestic livestock and poultry (Dryden et al., 1993). Over 400 arthropod-borne viruses (arboviruses) have been recognized, including the etiologic agents of such major livestock diseases as African swine fever, Akabane disease, bovine ephemeral fever, the equine encephalitides, bluetongue and epizootic hemorrhagic fever. These result in debilitation, lameness, blindness, wasting, congenital defects, abortions, sterility, and death in affected animals. Furthermore, arthropod-borne rickettsial agents, bacteria and blood protozoa cause several extremely significant livestock disease problems including tick-borne, Q, Mediterranean, and East Coast fevers, borreliosis, spirochetosis, tularemia, Lyme disease, babesiosis, theileriosis, and the trypanosomiases. The most prominent groups of arthropods that transmit these pathogenic agents to livestock are hematophagous and are biologically involved in transmission cycles e.g. ticks, tsetse flies, mosquitoes and biting midges. Of somewhat lesser importance are those hematophagous arthropod groups that mechanically transmit pathogens e.g. horse flies, deer flies, stable flies, horn flies (Committee on Foreign Animal Diseases of the United States Animal Health Association, 1998).

2. Factors limiting the efficacy of conventional agrochemical pesticides

To date, the major method of pest control has been the widespread use of classical organic agrochemical pesticides. These chemical pesticides were first introduced in the 1940's with the remarkable success of *p,p*'-dichlorodiphenyltrichloroethane (DDT). DDT was not only a successful agricultural pesticide, but was used in initially successful malaria eradication programs (Attaran et al., 2000) and is still in use in certain third world countries. The subsequent introduction of organophosphate and carbamate pesticides in the 1960s (Casida and Quistad, 1998) encouraged the customary view that chemical pesticides could produce widespread control of insect pests. However, the widespread use of classical agrochemical pesticides, along with their limited number of nervous system targets, has inevitably resulted in widespread resistance among arthropod populations.

2.1. Insecticide resistance

Despite this initial optimism, within one year of DDT being introduced for mosquito control in 1946 the first cases of DDT resistance occurred in two *Aedes* spp., a major malaria vector (Brown, 1986). Insect resistance is defined by the WHO as the 'development of an ability in a strain of an organism to tolerate doses of toxicant which would prove lethal to the majority of individuals in a normal (susceptible) population of the species'. Resistance to insecticides has now been reported in most major insect vectors. For example, in 1992 the WHO reported more than 500 species of insects and mites, including 56 anopheline and 39 culicine vector species, had developed resistance to one or more classes of insecticides (Georghiou, 1990; World Health Organisation, 1992) (Fig. 1B). Resistance has developed to every chemical class of insecticide, including microbial drugs and insect growth regulators. Insecticide resistance is expected to profoundly affect the re-emergence of vector-borne diseases (Krogstad, 1996), and where resistance has not contributed to disease emergence, it is expected to threaten disease control (World Health Organisation, 1992).

The underlying causes of insecticide resistance are many-fold. Due to the wide usage and narrow target range of agrochemicals, arthropods have been put under a high degree of selection pressure (Feyereisen, 1995; Brogdon and McAllister, 1998). Resistance can be characterised by (i) increases in metabolic detoxification resulting from elevated esterase, glutathione S-transferase or monooxygenase levels, (ii) decreased target sensitivity, and/or increased (iii) sequestration or lowered insecticide availability (Feyereisen, 1995; Brogdon and McAllister, 1998). The molecular mechanisms responsible for these increases in resistance have been identified as; point mutations in the ion channel of the GABA receptor, sodium channel and the acetylcholinesterase active site, amplification of the esterase genes, and mutations causing up-regulation of detoxification enzymes (Feyereisen, 1995; Brogdon and McAllister, 1998; Hemingway and Ranson, 2000).

Chemical insecticide control subjects insect populations to Darwinian selection and as a result, the development of resistance is inevitable. Moreover, the fact that the vast majority of insecticides in current use act on one of just five nervous system targets has promoted the development of cross-resistance to different families of insecticides (Brogdon and McAllister, 1998; Raymond-Delpech et al., 2005). These five target sites comprise: nicotinic acetylcholine receptors (cartap, spinosad, imidacloprid and related nitromethylenes/nitroguanidines), voltage-gated sodium channels (DDT, dihydropyrazoles, oxadiazines and pyrethroids), ©-aminobutyric acid receptors (cyclodienes, γ-BHC and fipronil), glutamate receptors (avermectins) and acetylcholinesterase (organophosphates and carbamates). Despite this, the world market for insecticides is still dominated by compounds that

inhibit the enzyme acetylcholinesterase (AChE). Together AChE inhibitors and insecticides acting on the voltage-gated sodium (Na_v) channel, in particular the pyrethroids, account for approx. 70% of the world market (Nauen et al., 2001). Importantly though the market share of AChE inhibitors decreased from 71% in 1987 to 51% in 1999. Given the impending use-cancellation of a range of AChE inhibitors the search for new insecticides and novel targets is becoming progressively more important.

2.2. Environmental and health impacts of insecticides

Selectivity of a chemical is achieved if both insect and non-pest species do not share the same target (Feyereisen, 1995). Unfortunately, because agrochemicals target sites conserved between insects and vertebrates, chemical pesticides have a relatively broad-spectrum of toxicity against non-target species, and thus potential widespread environmental effects. Natural predators of the targeted pest are also affected and there have been several examples where insecticide use has aggravated targeted insect-pest outbreaks (Winston, 1997). In addition, these chemicals can have equally toxic effects on non-target species such as crustaceans and fish, which are susceptible following spray drift, run-off or leaching into streams and rivers (for a review see (Van Wijngaarden et al., 2005).

A variety of acaricides and insecticides have also been shown to inhibit vertebrate mitochondrial respiratory complex I (Degli Esposti, 1998), implicated in the pathogenesis of movement disorders (Schulz and Beal, 1994). Importantly chronic, systemic inhibition of complex I by rotenone, causes highly selective nigrostriatal dopaminergic degeneration indicate that chronic exposure to certain pesticides can reproduce the neuropathological features of Parkinson's disease (Betarbet et al., 2000). Cohort and ecological studies also suggest a number of potentially adverse outcomes of pesticide exposure. More commonly suggested associations include pancreatic cancer, adverse reproductive outcomes, and neuropsychological dysfunction (Garabrant et al., 1992; Longnecker et al., 2001; van Wendel de Joode et al., 2001; Beard, 2006). While the evidence is weak, and the risk is low, these problems have potential to cause a significant increase in disease burden at a population level given the large number of people exposed to pesticides.

As a result of environmental and health concerns driven by the above issues, new U.S. federal regulations introduced in the 1990s place greater demands on insecticide risk assessment and registration. Together with U.S. federal mandated reviews of existing registered insecticides driven by the Food Quality Protection Act (FQPA), this has also led to de-registration, use-cancellation or abandonment of certain compounds for safety reasons or demanding re-registration requirements (Rose, 2001). In addition, some of the existing registrations for compounds on the market are over 45

years old and, due to resistance, many are not nearly as effective as when originally introduced. Thus, there is a critical need to identify new targets and isolate safe insecticides or lead compounds.

3. Development of biopesticides

Recent advances have highlighted the potential of genetic engineering in the development of novel (bio)insecticides. Bioinsecticides utilize natural organisms, or their products, in the production of transgenic plants or recombinant baculoviruses. In addition, the development of non-peptide mimetics based on naturally occurring insecticidal toxins is an avenue currently being considered.

3.1. Transgenic plants expressing insecticidal toxins

One approach, to reduce destruction of crops by phytophagous arthropod pests, is to genetically modify plants to express genes encoding insecticidal toxins. The production of transgenic plants that express insecticidal δ-endotoxins derived from the soil bacterium *Bacillus thuringiensis* (*Bt* plants) (see review by Bravo et al. this volume) were first commercialized in the U.S. in 1996. The expression of these toxins confers protection against insect crop destruction (Shelton et al., 2000). The lethality of *Bt* endotoxins is highly dependent upon the alkaline environment of the insect gut, a feature that assures these toxins are not active in vertebrates, especially in humans. These proteins have been commercially produced, targeting the major pests of cotton, tobacco, tomato, potato, corn, maize and rice, notably allowing greater coverage by reaching locations on plants which are inaccessible to foliar sprays (Shelton et al., 2000). This method of crop protection has obvious benefits because of its environmental and human safety, as well as a predicted lower cost (Shelton et al., 2000; Carriere and Tabashnik, 2001; Rowe and Margaritis, 2004). Despite dire predictions that the constant selection pressure will lead to resistance, there have been no documented reports for resistance to *Bt* crops in the field (Bates et al., 2005). Conversely, resistance has been documented for microbial *Bt* sprays in the field and laboratory (Tabashnik et al., 2004). Therefore, it is probably only a matter of time before resistance to transgenic crops develops.

3.2. Recombinant baculoviruses expressing insecticidal toxins

A transgenic approach that avoids introduction of foreign proteins directly into the food chain involves the application of insect-specific viruses engineered to express insecticidal neurotoxins (Cory et al., 1994). Importantly, baculoviruses are arthropod-specific, unable to infect vertebrates or plants (Herniou et al., 2003). Particular virus strains have limited host ranges and can infect one or a few species, with no effect on non-target arthropods (Black et al., 1997; Inceoglu et al., 2001). Although wild-type baculoviruses are highly specific for insects, and have been applied to protect crops in the

agricultural sector since the 1930s (Inceoglu et al., 2001) they are is limited by their slow 'time-to-kill'. From the application of the virus it will typically take days to weeks after infection for the insects to stop feeding, with consequent further damage to the crop (Bonning and Hammock, 1996). This shortcoming has been addressed by engineering recombinant baculoviruses that heterologously express insecticidal neurotoxin genes. Baculoviruses that express insecticidal toxins from mites (Tomalski and Miller, 1991; Popham et al., 1997), scorpions (McCutchen et al., 1991; Stewart et al., 1991; Cory and Hails, 1997; Gershburg et al., 1998; Sun et al., 2002), spiders (Prikhod'ko et al., 1996; Hughes et al., 1997; Prikhod'ko et al., 1998), sea anemones (Prikhod'ko et al., 1996; Hughes et al., 1997) and *Bt* (Chang et al., 2003) have all demonstrated a reduced time interval between virus application and cessation of feeding and/or death in three species of lepidopterans (Lu et al., 1996; Hughes et al., 1997; Prikhod'ko et al., 1998; Regev et al., 2003). Importantly, the natural infectivity and host range of the recombinant viruses did not appear to be altered by the introduction of heterologous toxin genes (Black et al., 1997). Although the speed of action has been greatly improved via the insertion of foreign gene products, the public reticence of the use of genetically modified organisms has contributed to their limited commercial development.

3.3. Peptidomimetics based on insecticidal toxins

Conformationally constrained peptides have been pursued as valuable tools in drug discovery and development, and could be applied in insecticide design. Theoretically, using a non-peptide organic scaffold, the peptide residues critical for binding to the target ("insectophore") can be grafted onto a backbone structure to produce a peptidomimetic. This provides a structure that topologically mimics the functional moieties corresponding to the insectophore. This non-peptidic analog has the potential to be used as a lead compound in the development of novel insecticides, overcoming the bioavailability issues of peptides penetrating the insect cuticle or gut mucosa. However, for rational insecticide design, one needs to know both the 3D structure and spatial position of the insectophore, information that is unfortunately lacking with most of the insecticidal toxins characterized to date. Nevertheless the concept has received limited validation following attempts to 'clone' the functional residues of peptide toxins that block vertebrate calcium or potassium channels (Menzler et al., 2000; Baell et al., 2001; Baell et al., 2002). The development of a peptidomimetic insecticide is likely to be challenging since non-critical residues determined in insect toxicity bioassays may be vital for averting vertebrate toxicity, via steric hindrance. In addition, these non-critical residues maybe important for providing insect target subtype selectivity.

4. Natural toxins as sources of insecticide leads

For the above approaches to be successful a wide range of characterized insect-selective toxins are required. Fortunately, the range of organisms that produce toxins for both defence, as well as for capturing arthropod prey, is surprisingly diverse. From an agrochemical perspective, the venoms of spiders, scorpions, and to a lesser extent, sea anemones, are of particular interest because of the remarkable diversity of toxins that are expressed in the venom glands of these animals. The venoms from these creatures are complex cocktails, which evolved primarily for the purpose of killing or paralyzing prey, and can potentially be exploited to identify leads for insecticide development. In addition, plants have evolved a range of compounds to protect against phytophagous pest attack and are also being considered. Importantly many of these organisms have, during their evolution, developed a complex pre-optimized combinatorial peptide library of neurotoxins, enzymes, antimicrobial and cytolytic peptides in their venom glands in the case of venomous animals or foliage, stems and roots of plants. This has enabled them to diversify their toxin pool for offence and/or defence mechanisms.

5. Scope of the special edition

There is an urgent need to isolate new and safe insecticidal lead compounds, validate new insecticide targets, and develop alternative methods of insect control. The aim of this special edition is to provide an overview of the range of toxins that potentially could be employed to achieve these goals. The following articles review several classes of insecticidal toxins from a diverse range of terrestrial and marine taxa from animal, plant and microbial sources that may fuel developments in each of these areas. Throughout this volume, articles also attempt to provide strategies of the delivery of these toxins as biopesticides.

The first two contributions review bacterial toxins with selective insecticidal activity. The first article by Bravo et al. deals with the pore-forming action of Cry and Cyt toxins from the bacteria *Bacillus thuringiensis*. In transgenic *Bt* plants expressing the Cry protein, toxin is produced continuously, protecting it from degradation and making it available to phytophagous insects. The development of transgenic crops these proteins has been a major break through as these insect resistant crops have considerably reduced the use of chemical pesticides in areas where these crops are planted. In their article, Bravo et al. compare the mode of action of Cry and Cyt toxins to other bacterial pore-forming toxins, detailing the multi-step process that involves the interaction with several receptor molecules leading to membrane insertion and cells lysis. The second article by ffrench-Constant et al. provides a thorough review of the array of toxins produced by *Photorhabdus* and *Xenorhabdus* bacteria and discusses their potential use in agriculture as alternatives toxins to *Bacillus thuringiensis*. Both

Photorhabdus and *Xenorhabdus* can penetrate the insect cuticle into the insect blood stream by their nematode vectors and produce a range of toxins with both oral and injectable insecticidal activity. This highlights the potential use of these vectors for the delivery of insecticidal toxins.

The next five contributions deal with insecticidal toxins derived from arachnid venoms. This animal taxon is currently receiving the most attention because many organisms from Class Arachnida rely on their venom to subdue, or kill, insect prey. Therefore, the venoms of these organisms are necessarily rich in insecticidal toxins–a fact already utilised in recombinant baculovirus constructs expressing various arachnid toxins that have successfully been field-trialed against lepidopteran pests (see section 5.2). The articles by Gurevitz et al. and Gordon et al. provide a thorough review of scorpion toxins that target ion channel gating and kinetics. Here the authors provide an in-depth appraisal of the extensive literature on both insect-selective α- and β-scorpion toxins detailing their sites and modes of action on site 3 and 4 of voltage-gated sodium channels. Both articles detail the bioactive surface of these scorpion toxins and provide key insights into the structural basis for their phyla-selectivity. The subsequent article by Nicholson focuses on similar, but structurally unrelated, peptide toxins from spider venoms. Here the range of insect-selective toxins appears to be wider with actions on at least three neurotoxin receptor sites but with a number of toxins at an early stage of characterisation. Therefore, less is known regarding the insectophore of these toxins. The article that follows by King focuses on the insect-selective spider toxins targeting voltage-gated calcium and potassium channel especially the ω- and κ-atracotoxins from funnel-web spider venom. The isolation of these novel atracotoxins highlights the advantage of screening for insecticidal activity rather than against known targets, and to validates these channels as novel insect targets. King discusses the potential of these toxins as bioinsecticides and as leads for the rational design of novel peptidomimetic insecticides. The last article in this section of the volume by Ushkaryov et al. covers insecticidal toxins from the highly venomous *Latrodectus* genus. Toxins found within *Latrodectus* venoms are different from the other arachnid toxins because they are composed mainly of high molecular mass latrotoxins of >100 kDa with unusual phyletic specificities (vertebrate vs. insect vs. crustacean). The authors provide a comparison of the five latroinsectotoxins with their structurally related vertebrate-selective counterpart α-latrotoxin. Their complex mode of action to stimulate neurotransmitter release is reviewed in detail along with the recent preliminary reconstruction of the three-dimensional structure of the δ-latroinsectotoxin monomer.

The article that follows by Bosmans et al. examines the insecticidal potential of marine toxins from sea anemones. These appear to be the only marine toxins that have been investigated for their

insecticidal potential with toxins from the sea anemones *Anemonia sulcata* and *Stichodactyla helianthus* being field trialled in a recombinant baculovirus model (Prikhod'ko et al., 1996). It is known that most sea anemone toxins act preferentially on crustaceans. Since crustacean and insect lineages are closely linked over evolution, it is not unexpected that these peptide toxins also modulate insect targets with phyletic selectivity over vertebrates. The authors review actions of sea anemone toxins on voltage-gated Na^+ channels in particular their actions on neurotoxin receptor site 3 to slow channel inactivation.

The penultimate article is by Gruber et al. who present an extensive overview of the cyclotides, plant defensive peptides, with unusual macrocyclic peptide backbones. These peptides are highly expressed in a variety of plants and have been shown to be toxic in feeding trials against *Helicoverpa spp.* pests. The review compares the structures and insecticidal activities of cyclotides with structurally similar cystine knot proteins from other plants. In addition, the authors discuss the potential of using the highly stable cyclic cystine knot motif as a scaffold upon which to graft various functions.

The final article by Whetstone et al. provides a timely and objective review of the various delivery methods currently in use for biopesticidal agents. The authors provide a overview of the sources of proteinaceous biopesticides including non-toxin derived neuropeptides and hormones and discuss various approaches to their delivery via oral ingestion or insect-selective infectious delivery agents such as bacteria, fungi, nematodes and viruses. The authors also examine the strengths and weaknesses of each method.

Obviously this special edition cannot cover all aspects of insect-selective toxins but the phyletic selectivity, range of approaches for delivery systems, and novel target validation reviewed in this volume provide impetus for further research. While biopesticides have yet to be considered as viable alternatives to classical agrochemical pesticides this volume provides clear evidence that an approach utilising insect-selective toxins has future potential for the control of pest arthropods. Issues such as cost, delivery methods, efficacy, target specificity, environmental persistence, and growth of pest resistance are factors that will all need to be considered in any future design of a cost-effective pesticide. Nonetheless, biopesticides clearly have a potential role to play in development of future integrated pest management strategies if we are to surmount disease transmission and crop destruction in the 21st century.

Graham M. Nicholson*

Tel.: +61-2-9514-2230; fax: +61-2-9514-2228.

Neurotoxin Research Group
Department of Medical & Molecular Biosciences
University of Technology, Sydney
PO Box 123, Broadway, NSW, 2007
Australia
E-mail address: Graham.Nicholson@uts.edu.au

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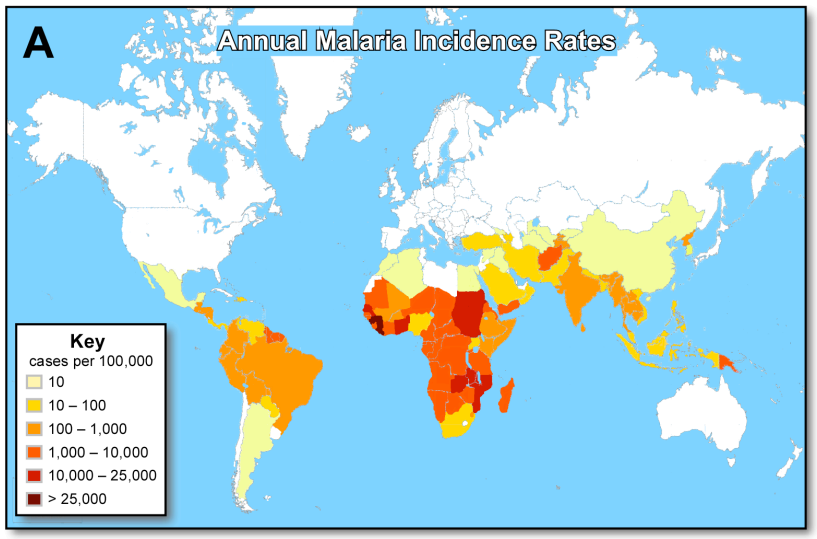
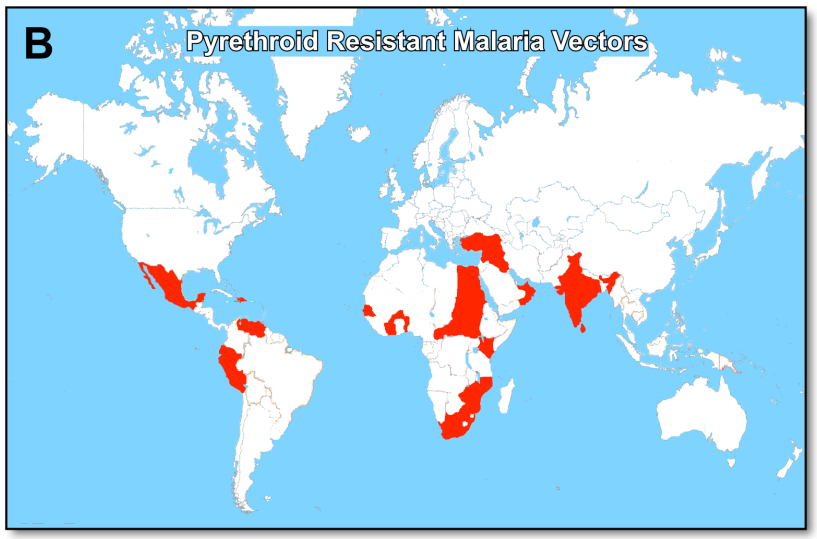
Figures


A
Annual Malaria Incidence Rates
Key
cases per 100,000
10
10 – 100
100 – 1,000
1,000 – 10,000
10,000 – 25,000
> 25,000


B
Pyrethroid Resistant Malaria Vectors

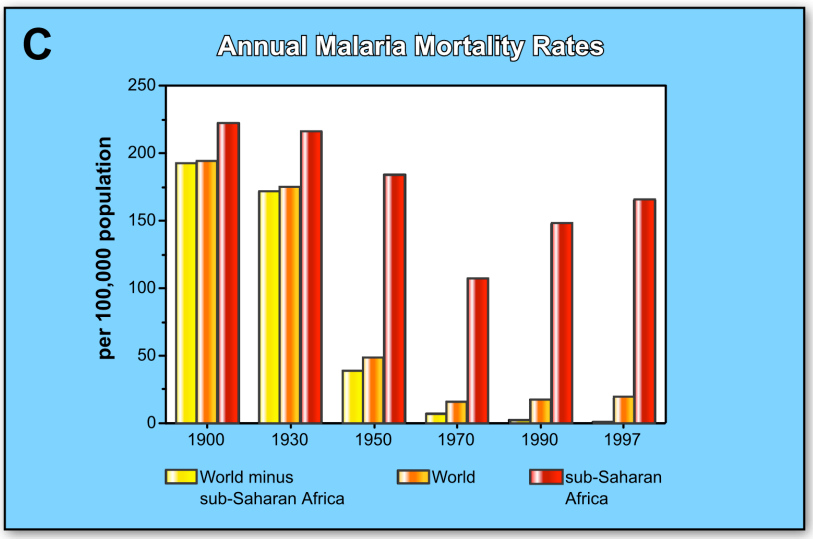

C
Annual Malaria Mortality Rates
per 100,000 population
250
200
150
100
50
0
1900
1930
1950
1970
1990
1997
World minus sub-Saharan Africa
World
sub-Saharan Africa

Fig. 1. Incidence and mortality of malaria. (A) Worldwide incidence rates for malaria per 100,000. Modified from the latest data available (January, 2004) from the World Health Organization. (B) Countries reporting malaria vector mosquito species with pyrethroid resistance (shading) (modified from (Zaim and Guillet, 2002). (C) Mortality rates from malaria per 100,000 population showing increases in malaria mortality rates in sub-Saharan Africa since late 1980s (World Health Organisation, 1999).