The venom of the Australian funnel-web spider is one of the most complex of any venomous animal, and is especially potent against a wide range of important insect pests.

Natural Insecticides from Spiders

Glenn King argues that insecticide chemists might do well to take lessons from spiders, the most efficient insect killers on the planet.

Insects are the most successful animals on the planet, with at least two million living species. However, their remarkable ability to inhabit virtually all terrestrial ecosystems has inevitably brought them into conflict with humans. Although only a small minority are classified as pests, they nevertheless destroy about 10% of the world's annual food production and transmit a diverse array of human pathogens. Mosquitoes alone cause over one million human deaths per year due to their ability to transmit the causative agent of malaria.

Insect pests have been controlled almost exclusively with chemical insecticides since the introduction of DDT in the 1940s. However, several developments over the past few decades have diminished the desirability of this chemical approach to insect control.

The publication of Rachel Carson's book *Silent Spring* in 1962 significantly raised public awareness of the adverse environmental impact of chemical insecticides, leading to a ban on the use of DDT and other chlorinated insecticides in the 1970s.

The long-term application of chemical insecticides also led to the evolution of resistance in many insect populations. This was an entirely predictable development, and it is analogous to the evolution of antibiotic resistance in bacteria. Over 500 species of insects are now resistant to one or more classes of chemical insecticides.

In thinking about new environmentally friendly approaches to insect pest control we noted that many of the most successful chemical insecticides were derived from natural products. The highly successful pyrethroid insecticides, for example, were derived from the natural pyrethrins found in chrysanthemum flowers. Thus we wondered what might be the best natural source of insecticidal compounds. The answer, in retrospect, was obvious.

Spiders are the best insect killers on

the planet. Despite their caricature as dark, hairy and dangerous, in reality they encompass an impressive variety of shapes, sizes, colours and predation strategies.

Moreover, in striking contrast to the large number of highly venomous snakes and scorpions, only four of the 40,000 characterised species of spiders are capable of fatally envenomating humans: the Australian funnel-web spider, recluse spiders, the Brazilian wandering spider and the red-back spider (which is known as the black widow spider in the United States). All other spiders, including the massive goliath tarantula with a leg span of up to 30 cm, are harmless to humans. In Australasia you have a significantly greater chance of being killed by a dog than a spider bite.

Many spiders engage in subterfuge in order to catch their prey or avoid predators. Crab spiders, for example, are clever invertebrate chameleons. They ambush bees and wasps by assuming the same colour as the flowers they frequent, thus camouflaging themselves from both predators and prey. Other species can mimic ants, beetles, wasps, twigs and even bird dung.

However, the primary predation tool for most spiders is an incredibly complex venom that is designed to rapidly kill or incapacitate their insect prey. Thus, our search for natural insecticides inevitably led us to a detailed investigation of the chemical composition of spider venom.

Work in my lab has focused on the venom of the Australian funnel-web spider for several reasons. First, this spider is one of the few from which venom can be milked without electrical stimulation of the venom apparatus, a process that often leads to contamination of the venom with digestive juices. When provoked, funnel-web spiders secrete venom onto their fang tips, which can then be collected with a pipette.

Second, like other primitive spiders such as tarantulas, funnel-web spiders are long-lived. Female funnel-web spiders can survive for more than 20 years. Thus we can maintain cohorts of female spiders in the laboratory for long periods of time, thereby ensuring a reproducible supply of venom.

Finally, our research has demonstrated that the venom of the Australian funnel-web spider is one of the most complex of any venomous animal, and is especially potent against a wide range of important insect pests.

Our studies have revealed that the venom of the Blue Mountains funnel-web spider *Hadronyche versuta* is comprised of more than 1000 different peptides. Peptides are small proteins, and those from the funnel-web spider are typically made up of 25–45 amino acids. However, these venom peptides are cross-braced by multiple disulfide bonds that give them a rigid three-dimensional structure and make them significantly more robust than most proteins. They are resistant to very high temperatures (>90°C), extremes of pH, as well as the action of proteases (enzymes that break down proteins).

One of these venom peptides, known as ∂-atracotoxin, is solely responsible for the deaths caused by Australian funnel-web spiders. This toxin prevents closure of the sodium channels that initiate electrical signals in the nervous system of both insects and humans. Curiously, this toxin is lethal only to insects and primates. Dogs and cats are completely immune to the bite of Australian funnel-web spiders, and funnel-web antivenom is produced by injecting the venom into rabbits.

Most importantly for our research, many of the remaining 1000 or so peptides in the venom of the Blue Mountains funnel-web spider are active against insects but are completely harmless to humans and other vertebrates.

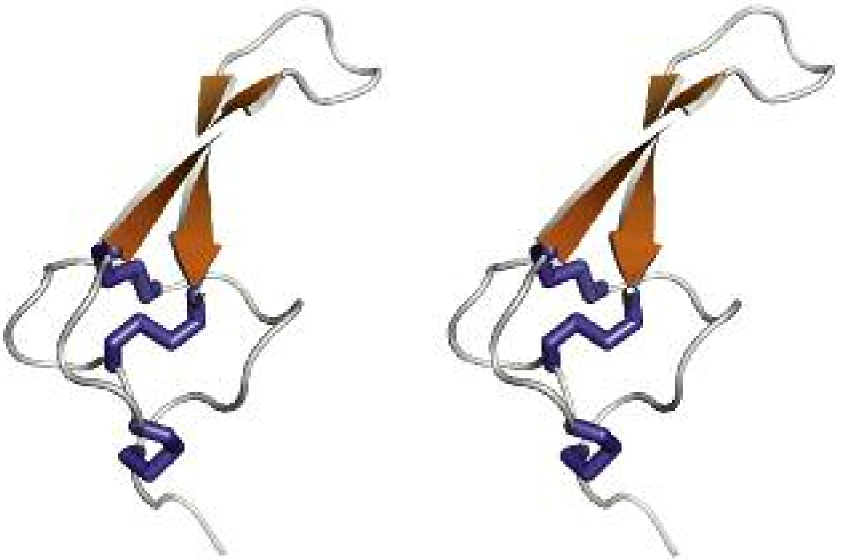
By carefully sorting through the many hundreds of peptides in funnel-web venom, we have isolated several groups of peptides that are lethal to a wide range of insect pests but are harmless to vertebrates. All of these peptides disrupt the activity of ion channels in the insect nervous system, but none of these ion channels are the target of current chemical insecticides.

Spiders have been working on the chemistry of insecticides for around 400 million years and they clearly have a much better "understanding" of the best ways to incapacitate insects than we do. Moreover, it appears that spiders evolved their diverse repertoire of venom peptides using a combinatorial chemistry approach, a technique that humans only "discovered" in recent decades.

Spiders use their two fangs, each of which resembles a small hypodermic

THE SPIDERS

Spiders evolved from an arachnid ancestor during the Devonian period around 400 million years ago, more than 150 million years before the appearance of dinosaurs. They are members of the taxonomic class Arachnida, which also includes scorpions, ticks and mites. Unlike insects, arachnids lack antennae and wings, and have eight legs rather than six. Whereas insects have three distinct body segments, most arachnids have only two: the cephalothorax and abdomen. The cephalothorax contains the eight legs, a pair of fang-bearing chelicerae in front of the mouth, and a pair of leg-like pedipalps located between the chelicerae and the first pair of legs. The spinnerets, from which silk is extruded through tiny spigots, are located at the back of the abdomen, which also houses the respiratory, circulatory, digestive and reproductive systems.



Stereoview of the three-dimensional structure of the spider toxin ω-atracotoxin-Hv1a. This peptide is composed of a linear array of 37 amino acids. The three disulfide bridges (blue) stiffen the peptide's structure and make it resistant to proteases. Focusing beyond the plane of the page yields a third central image that appears three-dimensional (similar to Magic Eye drawings).

needle, to inject their venom into insects. In all of our earlier experiments we replicated this approach by injecting venom peptides into insects in order to test their insecticidal activity.

The prevailing dogma in the field was that these peptides would be not be orally active because they would be degraded by proteases in the insect gut. Moreover, it was thought that even if the peptides survived the action of gut proteases, they would be incapable of traversing gut epithelial cells in order to reach the insect circulatory system (the haemolymph) and ultimately their molecular targets in the insect nervous system.

However, a surprise discovery led us to challenge and ultimately discredit this dogma. Most spider toxins act at peripheral neuromuscular junctions, but some of the peptides we discovered were found to act in the central nervous system, indicating that they are capable of breaching the insect's blood–brain barrier.

We therefore wondered whether these peptides could traverse other anatomical barriers, including epithelial cells in the insect gut. We subsequently found that several venom peptides were highly active when fed to the lone star tick *Amblyomma americanum*, which transmits a number of human pathogens, and the dengue mosquito *Aedes aegypti*.

The fact that these peptides are orally active opens up a number of opportunities for their deployment as "natural" insecticides. The peptides, which can be produced cheaply by bacterial fermentation, could be sprayed onto crops for insect control or used in baits to control insects such as ants, termites and cockroaches. Their lack of activity against vertebrate species, and the fact that they are broken down in the environment into natural amino acids, should make them a safe component of modern insect control strategies.

Moreover, the fact that these insecticidal peptides are genetically encoded mini-proteins means that they could also be expressed in crop plants to control insect pests. It has been shown, for example, that one particular insecticidal peptide from funnel-web spiders, ω-atracotoxin-Hv1a, provides excellent protection against two recalcitrant agricultural pests, the cotton bollworm *Helicoverpa armigera* and the cotton leafworm *Spodoptera littoralis*, when expressed in plants.

Our research represents only a small incursion into the mysteries of spider venom. We estimate that spiders as a group produce more than 10 million different insecticidal peptides, and that less than 0.1% of this amazing chemical diversity has been examined thus far.

It seems ironic that spiders, the iconic monsters of many B-grade horror movies, might ultimately provide an environmentally friendly solution to the control of insect pests.

Glenn King is Professor of Chemical and Structural Biology at The University of Queensland Institute for Molecular Bioscience.