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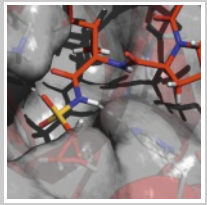

Medicinal Chemistry and Chemical Biology Highlights

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Peptides from Spider Venoms: A Natural Source of Bioinsecticides

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With the increasing global population and decreasing available arable land, there is a burden heavier than ever before on our ability to provide safe, nutritious and sustainable food. Therefore the control of insects, weeds and pathogens that harm agricultural production remains essential.[1,2] Arthropods and insects in particular damage $470 billion-worth of global crop production per year.[3] Annual crop yield lost to insects, currently 18–26% worldwide, is expected to increase in a warming climate.[4] Not only do arthropods threaten food production, they can also act as vectors transmitting deadly diseases.[5] The control of arthropod pests in both the agricultural and public health sector relies primarily on the application of chemical insecticides. Repeated use of commercial products has led to the development and global expansion of pest resistance.[6] Furthermore, there is growing public concern about the potential environmental and long-term human health impacts of certain agrochemicals.

Hence, the discovery of selective, effective and environmentally safe agrochemical alternatives to address the pest control challenge remains a necessity. While the crop protection market is dominated by small molecules, new modalities, such as silencing RNA,[7] microbial toxins,[8] and peptidic neurotoxins have received increased attention. Peptides in particular (defined as proteins less than 10 kDa) represent an appealing option as bioinsecticides, due to their potential to be highly potent, while showing exquisite species selectivity. Furthermore, being fully biodegradable into amino acids, peptides guarantee favorable environmental impact.

A great natural source of insecticidal peptides are the venoms of insect predators, *e.g.* spiders, scorpions, centipedes, wasps, predacious mites. Venoms used by insectivores to subjugate their prey are cocktails containing inorganic salts, small molecules such as biogenic amines, peptides and high molecular mass proteins, such as proteases.[9] Of particular interest for crop protection are the venom components that target receptors and ion channels in the insect nervous system.[10,11] An incredibly rich source of such insecticidal neuropeptides are spider venoms. ArachnoServer 3.0, a manually curated database of spider-venom peptides and proteins, contains to date >1500 peptide toxins from 100 spiders.[12] However, only a few are sufficiently potent to warrant consideration as bioinsecticides (*i.e.* LD50 < 1500 pmol g^{-1} by injection).[13] In addition to high intrinsic potency, there are several other requirements for a spider-venom peptide to be considered as a bioinsecticide lead, as summarized in Table 1.[14] Selectivity is crucial: ideally, a toxin should target only a narrow range of pest species while not harming vertebrates and other arthropods (*e.g.* pollinators and natural predators of the target pest species). This is the case of ω-Hexatoxin-Hv1a (ω-HXTX-Hv1a), a component of the Australian funnel web spider venom and one of the most potent insecticidal peptides known, which is harmless to vertebrates even at very high concentrations.[15] Importantly, ω-HXTX-Hv1a has also been shown to be non-toxic to bees, a strict requirement for modern insecticides.[16]

Criteria	Reason
Economical production	Costs need to be competitive with chemical insecticides currently on the market
No long-term persistence in environment	Prevent resistance development
Oral or topical bioavailability	Large scale insecticide application is usually by spraying or direct expression within plants
High potency and bioavailability	Saves material and thereby production costs
Selectivity towards target organism or non-lethal mode of action	Protects beneficial insects (*e.g.* pollinators) and vertebrates (*e.g.* livestock and humans)
Solubility/formulation	Should be easy to apply to achieve compliance by staff in the field
Stability under field conditions	Extremes in temperature, pH, UV radiation could lead to inactivation

Table 1 Desirable criteria (in alphabetical order) for the development of novel bioinsecticides. Reprinted from ref. 14, Copyright (2019), with permission from Elsevier.

Toxin size/complexity is also critical for bioinsecticide development: the higher the complexity, the more difficult it would be to economically produce large amounts of peptide for agricultural applications. The recent launch of Spear T® by the Vestaron Corporation (USA) provides proof-of-concept that spider venom peptides can go all the way to market and be manufactured on a large scale. The active ingredient of Spear T® is GS-ω/κ-HXTX-Hv1a, a spider venom-derived peptide commercialized as bioinsecticide for greenhouse use, targeting a wide range of insects.[17]

Nevertheless, with few exceptions, peptidic neurotoxins isolated from spider venoms are generally not orally active on insects. In contrast to most other peptides and proteins, stability is not a concern for these peptides as their particular fold, called a inhibitor cystine knot,[18] provides them with remarkable chemical and thermal stability as well as resistance to proteases.[9] The lack of oral insecticidal activity of venom peptides derives from the limited ability to traverse the gut epithelium to reach the target site, the nerves located in the insect hemocoel (body cavity). Spiders were not under evolutionary pressure to develop orally active peptide toxins, since they inject the venoms directly into the hemocoel of the prey. An array of strategies have been identified to significantly enhance the oral activity of venom peptides, in an attempt to allow their field application (Fig. 1).

One option is to modify the peptide chemically. Head-to-tail cyclization of ω-Hexatoxin-Hv1a has been performed in an aim to increase its oral potency, unfortunately without success.[19] Conjugation with polyethylene glycol polymers is another well-established approach to modify the properties of a peptide, but has not been applied to spider venom peptides, supposedly be-

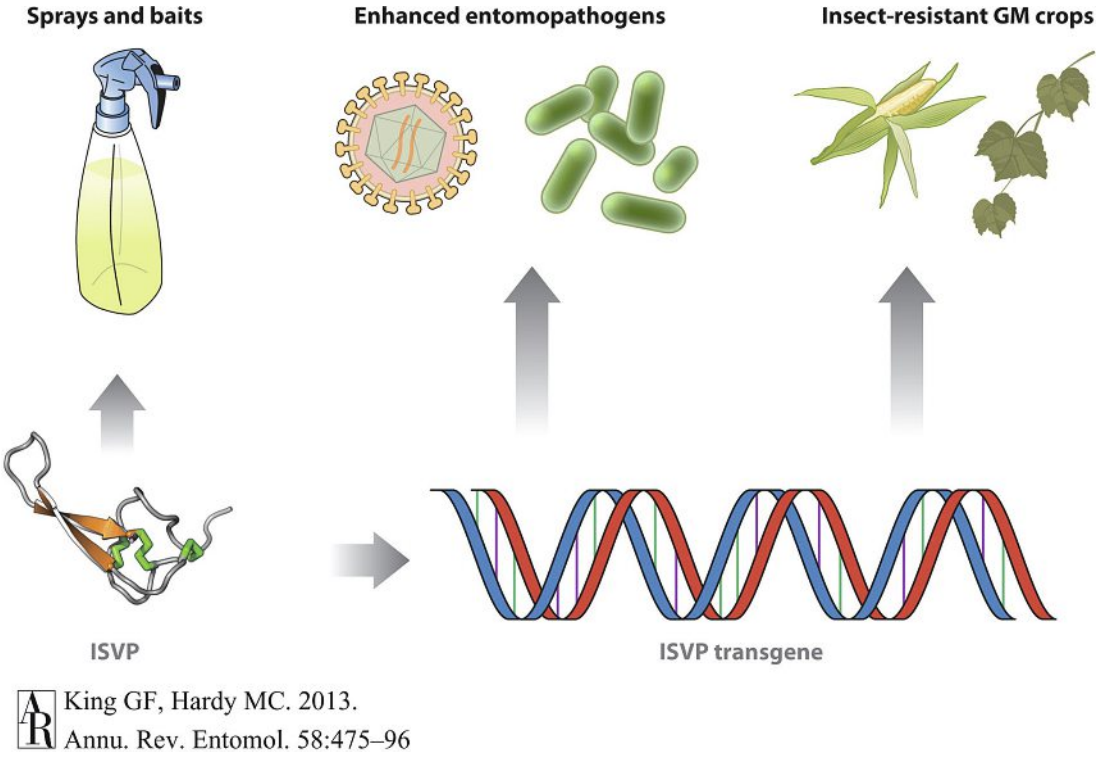

Fig. 1. Delivery options available for insect control with insecticidal spider-venom peptides (ISVP). Republished with permission of *Annual Reviews*, from ref. [9]. Copyright © 2013; conveyed through Copyright Clearance Center, Inc. Abbreviation: GM, genetically modified.

cause increased costs of manufacture and longer and costlier product registration can be expected for a semi-synthetic peptide. A promising alternative is fusion of the peptidic neurotoxin to a carrier protein that mediates its transport across the insect gut epithelium into the hemocoel. The mannose-specific lectin GNA (Galanthus nivalis agglutinin; snowdrop lectin) has been successfully used for this purpose. GNA binds to glycoproteins on the midgut membrane and has been shown to penetrate into the hemolymph whilst remaining intact. Enhanced gut translocation and improved oral activity of several insecticidal peptides was observed when these peptides were fused to GNA.[20]

A third option to improve oral efficacy of spider peptides is the use of entomopathogens as carriers.[21,22] The ability of engineered viruses or fungal pathogens to express venom toxins directly in the insect hemocoel has been successfully demonstrated. This approach further reduces the concerns regarding selectivity of insecticidal peptides toward beneficial insects, as the range of insects affected by the engineered toxin is naturally restricted to the host range of the pathogen.

Finally, another strategy to address the delivery of insecticidal spider peptides is the development of genetically modified (GM) crops encoding them. Transgenic tobacco expressing a neuropeptide from the Australian funnel-web spider was shown more than a decade ago to have enhanced resistance to arthropod pests.[23] Thus, ISVP transgenes could be regarded as stand-alone insect-resistant plant trait or as partners for trait stacking to minimize resistance development on GM crops.

The field of venom-derived insecticidal peptides is rapidly evolving, with exciting scientific developments. A new era of peptide-based bioinsecticides has just started.

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