Journal of Invertebrate Pathology 142 (2017) 1–4

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

The structure/function of new insecticidal proteins and regulatory challenges for commercialization

Genetically modified crops produced by biotechnology methods have provided grower benefits since 1995 including improved protection of crop yield, reduced input costs, and a reduced reliance on chemical pesticides (Klumper and Qaim, 2014). These benefits have driven annual increases in worldwide adoption of GM crops, with the largest number of hectares being grown in the Americas (James, 2014). In 2014, the majority of global biotech crops were planted to soybean [90.7 million (M) hectares), maize (55.2 M hectares) and cotton (25.1 M hectares). Herbicide tolerance and insect resistance traits are by far the most widely commercialized biotech traits. Of the 181.5 M hectares of biotech crops grown in 2014, approximately 43% (79 M hectares) contained insect resistance traits alone or stacked in combination with herbicide tolerance traits (James, 2014).

Among insect resistance traits, most commercial events are based on 3-domain crystalline (Cry) or vegetative insecticidal proteins (VIPs) from *Bacillus thuringiensis* (Bt) (Table 1). The long-term success of these traits has depended on the use of insect resistance management (IRM) strategies to delay insect resistance (Gould, 1998). Today there are several examples of insect pest populations that have evolved resistance to one or more Bt traits due to multiple generations of selection arising from deployment of these crops (Carrière et al., 2016). Field-evolved resistance to Bt proteins in crops such as maize and cotton requires new tools to manage the affected insect populations and continue to derive benefits from these Bt crops. One approach to counter insect resistance to single traits is to combine (pyramid) two or more proteins with differences in their mechanisms of action that are effective against the target pest(s) (Roush, 1998). For example, SmartStax® maize was the first pyramided Bt crop offering protection using two distinct mechanisms of action (Cry3Bb1 and Cry34Ab1/Cry35Ab1) against the western corn rootworm, *Diabrotica virgifera virgifera*.

Classes of proteins that are not susceptible to cross resistance with currently commercialized insect resistance traits, and/or control other pests not controlled by current products, are needed. Table 2 depicts some of the non-3-domain insecticidal proteins currently in various stages of trait development.

The 3-domain group of insecticidal Cry proteins has been the subject of extensive study over many years, including the first structure that was published in 1991 (Li et al., 1991). In contrast, our knowledge of non-3-domain toxins is far less advanced. Understanding of the mechanisms of action of these new families of insecticidal proteins will be greatly facilitated by elucidation of their structures. Knowledge of structure and function may allow toxin modification to modulate and retarget their activity, help to delay resistance development to existing traits, and also contribute to predictions of their specificity (target pests and non-target species) that can be validated through experimental testing. Recent advances in this field have increased the number of non-3-domain protein structures available, thus improving our understanding of the relationship between structure and function, resulting in a more knowledge-based prediction of activity. Nonetheless, major challenges remain.

This Journal of Invertebrate Pathology Special Issue is primarily a compilation of manuscripts from two meetings of the Society for Invertebrate Pathology (SIP) that aimed to assess the current state of the art in structure, function and commercial development of non-3-domain proteins. Papers arising from these meetings are presented here to make them available to a wider audience and to suggest directions for further research to advance the field. Papers are derived from a symposium at the 2014, 47th Annual SIP meeting in Mainz, Germany organized by Ken Narva and Colin Berry: "Structure and Function of Novel Insecticidal Toxins", followed by a complementary workshop at the 2015 International Congress on Invertebrate Pathology and Microbial Control, and the 48th Annual SIP meeting in Vancouver, British Columbia, Canada organized by William Moar and Ken Narva: "Regulatory Considerations for the Commercialization of New Insecticidal Proteins". An overview of the presentations is shown in Table 3, below.

The goal of the 2014 symposium was to discuss new information on the structure and function of new insecticidal proteins while the 2015 workshop built on the 2014 symposium and discussed how knowledge of the structure/functions of new insecticidal proteins can address various topics (primarily non-target safety) required for regulatory approval. Since the 2014 symposium and 2015 workshop, a symposium entitled "Novel Insecticidal Agents and Next Gen Approaches for Insect Control was held at the 2016 International Congress of Entomology Conference in Orlando, Florida representing the next in a series of ongoing, global scientific discussions on new insecticidal proteins, the purpose of which was to share the state of the art of the technology, promote further research, and to assess and promote safe uses of the technology. Given the increasing number of insect resistance traits with elucidated protein structures we anticipate this area of research to be actively discussed in future meetings such as SIP.

Since the 2014 SIP Symposium, peer-reviewed manuscripts have been published demonstrating (1) numerous new insecticidal proteins are being developed to control insect pests and (2) their elucidated structures and integrating this structural information with biochemical and bioinformatic analyses can enable testing and identification of structural and functional domains responsible

http://dx.doi.org/10.1016/j.jip.2017.02.001
0022-2011/

Table 1
Maize events expressing *Bacillus thuringiensis* Cry and VIP insecticidal proteins commercialized in the U.S.

Developer	Event Name	OECD Unique Identifier	*Bt Protein(s)*	Pest Spectrum	Year Approved (Cultivation - USA)	Non-IR Genes[a]
Syngenta	176	SYN-EV176-9	Cry1Ab	Lepidoptera	1995	*pat*
Monsanto	MON 810	MON-00810-6	Cry1Ab	Lepidoptera	1996	*nptII*
Syngenta	Bt11	SYN-BT011-1	Cry1Ab	Lepidoptera	1996	*pat*
Dekalb Genetics Corporation	DBT418	DKB-89614-9	Cry1Ac	Lepidoptera	1997	*bar*
Aventis CropScience	CBH-351[b]	ACS-ZM004-3	Cry9C	Lepidoptera	1998	*pat*
Dow AgroSciences DuPont Pioneer	TC1507	DAS-01507-1	Cry1Fa	Lepidoptera	2001	*pat*
Monsanto	MON863	MON-00863-5	Cry3Bb1	Coleoptera	2003	*nptII*
Dow AgroSciences DuPont Pioneer	DAS-59122-7	DAS-59122-7	Cry34Ab1 Cry35Ab1	Coleoptera	2005	*pat*
Monsanto	MON88017	MON-88017-3	Cry3Bb1	Coleoptera	2005	*cp4 epsps*
Syngenta	MIR604	SYN-IR604-5	mCry3A	Coleoptera	2007	*pmi*
Monsanto	MON89034	MON-89034-3	Cry1A.105 Cry2Ab	Lepidoptera	2008	
Syngenta	MIR162	SYN-IR162-4	Vip3Aa20	Lepidoptera	2010	*pmi*
Syngenta	5307	SYN-05307-1	eCry3.1Ab	Coleoptera	2012	*pmi*

[a] Non-IR (insect resistance) Genes *pat*: a selectable marker which confers tolerance to the herbicide glufosinate ammonium in plant tissue. *nptII*: a selectable marker which confers the ability to metabolize the antibiotics neomycin and kanamycin in plant tissue. *bar*: a selectable marker that confers tolerance to the herbicide glufosinate ammonium in plant tissue. *cp4 epsps*: a selectable marker confers tolerance to the herbicide glyphosate in plant tissue. *pmi*: a selectable marker that confers the ability to utilize mannose as a carbon source in plant tissue.
[b] Approved for environmental release and use as animal feed only.

Table 2
Examples of non-3 domain insecticidal proteins in various stages of trait development.

Developer	Protein name	Protein structure family	Source	Pest spectrum	Reference
Monsanto	Cry51Aa2.834_16	Beta pore forming protein	*B. thuringiensis*	Hemiptera	Gowda et al. (2016)
Monsanto	TIC 2463	Beta pore forming protein	*B. thuringiensis*	Coleoptera	US20150274786
Dow AgroSciences	Cry6Aa	Alpha helical pore forming proteins	*B. thuringiensis*	Coleoptera	Dementiev et al. (2016)
Bayer Crop Sciences	GNIP1Aa	Membrane attack complex/perforin (MACPF) superfamily	*Chromobacterium piscinae*	Coleoptera	This issue
DuPont Pioneer	PIP-72Aa	Unknown	*Pseudomonas chlororaphis*	Coleoptera	WO2015/038,734
DuPont Pioneer	AfIP-1A, AfIP-B	AfIP-1A: Aegerolysin PFAM	*Alkaligenes faecalis*	Coleoptera	US20140033361
Monsanto	TIC 3670	Beta-pore forming protein	*Brevibacillus laterosporus*	Coleoptera	US20160319302

Table 3
Structure/function presentations at the 2014 and 2015 SIP conferences.

2014 SIP Conference Symposium:
Structure and Function of Novel Insecticidal Toxins
Organizers/Moderators: Ken Narva and Colin Berry
1. **Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1**
   Matthew S. Kelker, Colin Berry, Matthew D. Baker, Steven L. Evans, Reetal Pai, David McCaskill, Joshua C. Russell, Nick X. Wang, J.W. Pflugrath, Cheng Yang, Matthew Wade, Tim J. Wess, Kenneth E. Narva
2. **Structure/function studies of Cry5B via alanine scanning mutagenesis**
   Jillian Sesar; Melanie Miller, Yan Hu, Raffi V. Aroian
3. **Insights into the structures of non-3-domain toxins through structural modelling**
   Colin Berry
4. **Novel MTX Toxins for Insect Control**
   Yong Yin
5. **Insecticidal toxins from *Photorhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins**
   Thomas Jank, Alexander E. Lang, Klaus Aktories
6. **Molecular basis of parasporin-2 action toward cancer cells**
   Sakae Kitada, Yusuke Yoshida, Yoshimi Ozaki, Hirioyasu Shimada

2015 SIP Conference Bacteria Division Workshop:
Regulatory Considerations for the Commercialization of New Insecticidal Proteins
Organizers/Moderators: William Moar and Ken Narva
1. **Current insights on Bt insecticidal protein specificity and future direction**
   Juan Luis Jurat-Fuentes, Neil Crickmore
2. **Proteins 101: structure, function, and evolution**
   Joe Jez
3. **Protein sequences, structures and functions: rules for divergence and rules for conservation**
   Adam Godzik
4. **Modelling of insecticidal toxins and their potential interactions: Challenges and aspirations**
   Colin Berry, Neil Crickmore
5. **Safety considerations derived from Cry34/35Ab1 structure and function**
   Kenneth E. Narva, Nick Storer, Rod Herman