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PLANT SCIENCE SCAN

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BACKGROUND: The Plant Health Science Division of the Canadian Food Inspection Agency routinely scans external sources to identify information that might be of possible regulatory significance or interest to Canada's national plant health. This Plant Science Scan report was prepared by the Canadian Food Inspection Agency's staff as a mechanism to highlight potential items of interest, raise awareness and share significant new information related to plant health.

Index of Articles

Numbered squares correspond to numbered articles above

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canada

Pathology

1 First report of carrot cyst nematode, *Heterodera carotae*, in Canada

Recently, the carrot cyst nematode, *Heterodera carotae* Jones, was isolated from cultivated carrot (*Daucus carota*) roots in the Holland Marsh region, Simcoe County, Canada. This is the first report of *H. carotae* in Canada. Affected plants exhibited stunted growth, dwarfed and forked primary roots, proliferation of secondary roots, and root cysts. The damage attributed to carrot cyst nematode was high (>35%) in some sections of the two investigated fields. *Heterodera carotae* is only known to infect carrots and wild carrots and has been reported from parts of Europe, Russia, India, and the state of Michigan in the USA. Ontario is the largest producer of carrots in Canada; the distribution and potential for damage of this nematode is not known but a survey by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), the University of Guelph and Agriculture and Agri-Food Canada (AAFC) is in progress.

Heterodera carotae is currently not a regulated pest for Canada. Symptoms of *Heterodera carotae* have been observed in Holland Marsh region for a number of years and therefore, the nematode may have a wider distribution. It appears that this nematode may not meet the definition of a quarantine pest as defined by the International Plant Protection Convention (IPPC).

SOURCE: Yu, Q., E. Ponomareva, D. Van Dyk, M. R. McDonald, F. Sun, M. Madani, et al. (2017) First report of the carrot cyst nematode (*Heterodera carotae* Jones) from carrot fields in Ontario, Canada. Plant Disease DOI: 10.1094/PDIS-01-17-0070-PDN from http://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-01-17-0070-PDN.

2 First report of blackleg of potato caused by *Dickeya solani* in Brazil

Recently, blackleg of potato caused by *Dickeya solani* was isolated from a commercial field in Minas Gerais, Brazil. A high incidence of blackleg and soft rot symptoms were observed on potato (*Solanum tuberosum* cv. Taurus) originating from a contaminated lot of seed tubers. Presence of the bacterium was confirmed using morphological, biochemical and molecular tests and identification was confirmed using Koch's postulates. This is the first report of *D. solani* in South America.

Dickeya solani has been considered an economically important plant pathogen of potato and the pathogen also infects *Hyacinthus orientalis* L. Since its first detection in Poland in 2005, *D. solani* has spread rapidly across Europe and is considered the most significant causal agent of blackleg disease on potato in the region. Until now this pathogen has not been detected in the western hemisphere. *Dickeya solani* is not currently a regulated quarantine pest in Canada but introduction of this pathogen is of serious concern to the potato industry. The ability of this pathogen to adapt to changing climatic conditions and the economic impact the disease can cause makes this a pest of potential quarantine significance.

SOURCES: Cardoza, Y. F., Duarte, V. and Lopes, C. A. (2017) First report of blackleg of potato caused by *Dickeya solani* in Brazil. Plant Disease 101(1): 243.

Golanowska, M. and Lojkowska E. (2016) A review on *Dickeya solani*, a new pathogenic bacterium causing loss in potato yield in Europe. Journal of Biotechnology, Computational Biology and Bionanotechnology 97 (2): 109-127.

3 First report of white root rot caused by *Rosellinia necatrix* on *Aronia melanocarpa* in Korea

In July 2015, black chokeberry (*Aronia melanocarpa* cv. Nero) shrubs were found to be exhibiting symptoms of white root rot. The shrubs were sampled from an orchard in Gochang, Korea. Plants showed approximately 5% disease incidence with typical symptoms of white root rot including leaf yellowing and wilting. *Rosellinia necatrix* was identified based on morphological, molecular and pathogenicity analyses. This is the first report of *R. necatrix* on *A. melanocarpa* (new host) and a first record of this pathogen in Korea (new location). The shrub, *Aronia melanocarpa,* is a popular ornamental and native plant in Canada. *Rosellinia necatrix* is a quarantine pest for Canada and phytosanitary import requirements are in place for *Vitis* spp.

SOURCE: Choi, I. Y., Oh, H. T., Lee, W. H., Cho, S. E. and Shin, H. D. (2017) First report of white root rot caused by *Rosellinia necatrix* on *Aronia melanocarpa* in Korea. Plant Disease 101(1):253.

4 Genomic analysis of a recently identified onion pathogen, *Pantoea allii*

Pantoea allii is a relatively new pathogen causing leaf blight, necrosis and bulb decay in onion in South Africa and the USA. This species is phylogenetically most closely related to *P. ananatis* and *P. stewartii*. Due to phenotypic similarities, accurate identification of these species depends on species-specific genetic variation. In order to generate new knowledge and develop reliable and rapid diagnostic tools for *P. allii*, a research team at Agriculture and Agri-food Canada (AAFC) and Carleton University recently sequenced the genome of this new pathogen. Analysis of the first genome assembly of *P. allii* DOAB 206, the strain previously isolated from onion in South Africa, revealed that the genome is about 5.2 Mb with 4880 protein-encoding sequences and a G+C content of 52.9%, which is in keeping with the DNA G+C content of the genus *Pantoea*. Digital DNA-DNA hybridization revealed a 35.7-36.5% similarity between *P. allii* and the closest phylogenetic relative, *P. ananatis*. Pathogenicity tests showed that *P. allii* can infect Canadian bulb and green onions.

Leaf blight, leaf rot, bulb rot and bulb decay of onion caused by *Pantoea ananatis* and *P. agglomerans* have been reported in major onion and garlic production areas in Canada; there is no report that the new species, *P. allii*, is more virulent than these two species.

SOURCE: Hsieh, S., Xu, R., Avis, T. and Tambong, J.T. (2016) Genome analysis and pathogenicity of a new potential biothreat, *Pantoea allii*, to onion production in Canada. Poster presented at the 87th Canadian Phytopathological Society Meeting; 2016 June 12-15; Moncton, NB. Abstract # P16, page 45.

Entomology

5 Investigation of survey tools for *Agrilus* species associated with beech and poplar in Slovakian forests

Woodborers in the *Agrilus* genus (Coleoptera: Buprestidae) pose a high invasive potential due to the sheer number of species, their xylophagous feeding niche associated with pathways of introduction and widespread polyphagy. The variable effects of trap colours and volatile treatments across *Agrlius* species and sexes make it essential to document the response of adults to various trap types and designs.

In 2013 and 2014, field bioassays were conducted in Slovakian beech (*Fagus* spp.) and poplar (*Populus* spp.) forests to evaluate the response of adult *Agrilus* to green and purple sticky prism traps baited with various lure treatments (blank, cubeb oil or (*Z*)-3-hexenol) (Rhainds et al., 2017). The two most abundant species detected during the study were *A. viridis* in beech forest (146 adults, >95% females) and *A. convexicollis* in poplar forest (158 adults, two-thirds males). The two species exhibited opposite responses to trap colour as purple traps attracted 2-3x more *A. viridis* than green traps, and >95% of *A. convexicollis* were captured on green traps.

In Canada, the geographic expansion of emerald ash borer (EAB), *A. plannipennis,* is currently monitored using green prism traps baited with (*Z*)-3-hexenol and (*Z*)-3-lactone lures. The high number of adult *A. convexicollis* captured on green prism traps observed during the study by Rhainds et al. (2017) implies that traps used to monitor EAB could also be used to detect introductions of *A. convexicollis*. The high number of *A. viridis* females captured on purple prism traps baited with cubeb oil suggests that this combination could be used as a survey tool for detection of female *A. viridis*. The results also demonstrate that proactive research in the native range of potential invaders can benefit the development of monitoring protocols before invasion takes place.

SOURCE: Rhainds, M. Kimoto, T., Galko, J. Nikolov, C. Ryall, K, Brodersen, G. and Webster, V. (2017) Survey tools and demographic parameters of Slovakian *Agrilus* associated with beech and poplar. Entomologia Experimentalis et Applicata 162: 328-335 DOI: 10.1111/eea.12546

6 Broadleaf trees of questionable suitability for Asian longhorned beetle, *Anoplophora glabripennis*, development in Canada

In 2003, the Asian longhorned beetle (ALHB), *Anoplophora glabripennies* (Coleoptera: Cerambycidae) was discovered in an industrial area at the boundary of Toronto and Vaughan in Ontario, Canada. The discovery prompted the Canadian Food Inspection Agency (CFIA) to delineate a regulated area (152km^2) and implement an eradication program. Infested trees and trees of 10 genera considered suitable hosts within 400m of an infested tree were removed as part of eradication efforts. A tree inventory in the regulatory area revealed the presence of several tree genera whose suitability for ALHB development was either unknown or questionable from records that were unverified under field conditions. Since ALHB will continue to pose a significant risk to Canadian trees for the foreseeable future, it is important to know which tree species are suitable for the complete development of ALHB, and also to ensure that genera not officially targeted for survey or treatment are indeed not suitable.

To acquire knowledge on host plant selection and development of ALHB in Canada, a study by Turgeon et al (2016) surveyed over 3000 trees from genera with unknown or questionable suitability for three years following the removal of infested trees in the regulated area. During the survey, it was found that none of the trees in the 18 genera that were examined were attacked, except for one ash (*Fraxinus excelsior*) tree which had signs of oviposition and early-instar development, but not of adult emergence. Before this survey, an earlier survey in 2003 found a single little leaf linden (*Tilia*

cordata) with many signs of injury, but no signs of larval survival or emergence. These findings suggest that *F. excelsior* and *T. cordata* are resistant or unsuitable for ALHB development, at least in Ontario. The authors note that colonization of unsuitable trees may occur where population pressure is high. They nonetheless recommend that future eradication programs continue to allocate resources to assess and report information pertinent to attack on species from these genera in order to support successful eradication programs.

SOURCE: Turgeon, J.J., Jones, C., Smith, M.T., Orr, M., Scarr, T.A. and Gasman, B. (2016) Records of unsuccessful attack by *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on broadleaf trees of questionable suitability in Canada. Canadian Entomologist 148: 569-578.

7 Collecting host-marking pheromone from blueberry maggot, *Rhagoletis mendax,* to explore potential applications as a pest management tool

The blueberry maggot, *Rhagoletis mendax* (Diptera: Tephritidae), is an important pest of commercial lowbush and highbush blueberries in many parts of eastern North America. Females of *R. mendax* use a host-marking pheromone (HMP) after they oviposit in fruit, to deter further oviposition from conspecific females. The authors of this study investigated the collection of HMP from *R. mendax* to determine its usefulness as a potential pest management tool. Specimens for study were collected as pupae from lowbush blueberry fields in Nova Scotia, Canada. The HMP was collected from the faeces of emerged adult flies and from artificial oviposition devices. Mated *R. mendax* females were selected for two-choice bioassays that contained domes with a faeces extract, a crude pheromonal solution (from the oviposition device), and an untreated solution (control). The authors found that females showed a significant preference for oviposition in domes treated with the control solution versus domes treated with extracts from either of the two HMP sources. This result provides evidence that the HMP could be an effective control measure used against this pest. In addition, the thorough methodology of the collection and extraction of HMP from fly faeces and artificial oviposition devices presented in this study is a significant contribution towards the development of pest management tools for *R. mendax*, and potentially for other *Rhagoletis* species as well.

Rhagoletis mendax is a regulated quarantine pest for Canada, present so far in Prince Edward Island, Nova Scotia, New Brunswick, and small isolated populations that have been detected in southwest Ontario and southwest Québec. Further advances in management strategies for this pest will help provide alternative controls that can be used in blueberry orchards, such as reducing potential chemicals sprays used against this pest.

SOURCE: Faraone, N., Hillier, N. and Cutler, G. (2016) Collection of host-marking pheromone from Rhagoletis mendax (Diptera: Tephritidae). The Canadian Entomologist, 148(5): 552-555. DOI:10.4039/tce.2015.87

8 Ability of tomato leafminer, *Tuta absoluta*, to develop on wild and cultivated solanaceous plants

The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), is an invasive species that is highly destructive to tomato (*Solanum lycopersicum*) plants and fruits. During the past decade the pest has spread from its native range in South America to areas of Central America, North Africa, Asia and Europe, posing a widespread threat to commercial tomato production. Although *T. absoluta* has been reported on other economically important solanceous crops and wild plants, little is known on

its effective development and reproductive capacity on these alternative hosts.

A recent study investigated the ability of *T. absoluta* to locate and develop on wild (*Atropa belladonna, Datura stramonium, S. nigrum*), and cultivated (*S. tuberosum*) solanaceous plants to determine whether these species could act as refuges or reservoirs for the leafminer (Bawin et al., 2015). Dual-choice behavioural assays performed in flying tunnels demonstrated that females preferentially oriented to *Solanum* species over the wild *Solanaceae* tested. However, it was noted that oviposition occurred on each of the tested plants suggesting that all plants could be identified as potential hosts for offspring development. Fitness assays by rearing larvae on each plant species demonstrated that *Solanum* species supported higher larval survivability and lower development time compared to the other plants tested.

The results of this study suggest that other solanaceous plants, such as the ubiquitous common nightshade (*Solanum dulcamara*) are also potential hosts even though others in the same family are not (e.g. tobacco, *Nicotiana rustica*). Common nightshade may be a host, but this is uncertain: no females reared on it laid eggs, and not enough sampling was done to determine whether the complete loss of female fecundity when reared on nightshade extends to the natural condition. This leaves open the question whether there are any native or escaped plants in Canada that are suitable alternate hosts. In cultivated areas, it is likely that sufficient hosts exist.

Tuta absoluta is a regulated pest for Canada of great concern due to challenges with eradication from greenhouses that continually produce solanaceous crops.

SOURCE: Bawin, T., Dujeu, D., de Backer, L., Francis, F. and Verheggen, F. J. (2016) Ability of *Tuta absoluta* (Lepidoptera: Gelechiidae) to develop on alternative host plant species. Canadian Entomologist 148(4):434-442.

9 New tools for monitoring e-commerce trade in invasive plants

Global internet trade (e-commerce) is increasingly recognized as a major pathway for the introduction of non-native plant species to new areas. Online trade offers easy shopping for consumers and new market opportunities for sellers; horticultural enthusiasts now have access to a wide range of plant species from a global network of suppliers in a competitive virtual marketplace that is very difficult to monitor and often bypasses border controls and plant health regulations. Several organizations are developing new software and automated search algorithms to improve their ability to monitor e-commerce in plants and plant products.

In 2015 a group of Swiss researchers developed a software tool that systematically downloaded information on internet offers of over 150,000 pre-defined plant species on eBay.com. They found 2625 species offered for sale during a 50 day search, of which 510 were identified as invasive species. Of the 100 most frequently offered species (many offered on a daily basis), a major proportion were ornamentals (64%), sometimes in combination with other uses (e.g., medicinal), and almost half were invasive species (41%). Offers originated from 65 different countries, with most vendors offering to ship anywhere in the world.

This study focussed on one site representing a small fraction of the total internet trade in invasive plants, and showed that international horticultural e-commerce is not yet effectively regulated.

More recently, the North American Great Lakes Commission (GLC) developed a similarly innovative software program to monitor internet trade in aquatic invasive species in the Great Lakes region, called The Great Lakes Detector of Invasive Aquatics in Trade (GLDIATR). This open source, customizable software uses natural language processing and machine intelligence to search thousands of pages across the internet for keywords including the scientific and common names of species. In its first 30 days of full-scale testing, GLDIATR scanned over 300,000 web pages and identified 200 websites with aquatic invasive species for sale, including 56 species that are regulated in one or more states or provinces bordering the Great Lakes. The GLC used the information to contact website owners with information about invasive species regulations and encourage best practices. They subsequently observed changes to stock and/or shipping restrictions in many cases.

These studies highlight the fact that e-commerce presents a significant challenge to current biosecurity programs, and that automated algorithms used in software such as GLDIATR could play an important role in the future. They can be used to gather information on trade patterns, for horizon scanning, and for early detection of new species, helping to focus regulatory and voluntary compliance activities and ultimately helping to prevent the spread of invasive species. The adaptable and open source nature of GLDIATR make it an interesting option for other organizations looking to adopt similar technologies.

SOURCES: Great Lakes Commission (2017) Internet trade of aquatic invasive species. [Online] Available: http://www.glc.org/work/gldiatr (accessed March 2017).

Humair, F., Humair, L., Kuhn, F. and Kueffer, C. (2015) E-commerce trade in invasive plants. Conservation Biology 29(6): 1658-1665. DOI: 10.1111/cobi.12579.

Jensen, E. (2017) GLDIATR: Protecting the Great Lakes from internet trade of AIS. Presentation at Canada's 3rd National Invasive Species Forum, Ottawa, ON, February 28-March 2, 2017. [Online] Available: http://canadainvasives.ca/news-events/save-the-date-3rd-national-invasive-alien-species-forum/speakers/ (accessed March 2017).

10 National parks invasive plant watch lists

The national parks of Canada are a source of pride for Canadians and an integral part of our identity. Located in every territory and province, they represent and celebrate the beauty and variety of Canada's ecosystems. Parks Canada is the government agency responsible for protecting and preserving the unique ecological integrity of each park for current and future generations. Invasive species present a challenge for the management parks due to their negative impacts on biodiversity and ecosystem processes. Collaborations between national parks and plant protection agencies can contribute to the management of parks by preventing invasive species introductions in these highly valued environments.

A recent study in the U.S. demonstrated how risk assessments were used to create an invasive plant watch list for national capital region parks, to help resource managers prioritize efforts for those species most likely to impact their parks. The author first developed a candidate list of 97 species of concern by querying invasive plant records within a 241 km (150 miles) radius of the parks from the Early Detection and Distribution Mapping System (EDDMapS), removing those species determined to be native or already present in the parks. The watch list was then further refined to 20

species by evaluating all candidate species with a risk assessment process and prioritize those species assigned a 'high' impact ranking. This watch list will be valuable for park managers, to guide identification training and inform surveillance and early detection efforts for these critical invasive plant species that have a high likelihood of being found in the parks in the near future.

In a similar exercise in Canada, the CFIA recently collaborated with Parks Canada to create preliminary invasive plant watch lists for two national parks, namely Bruce Peninsula (ON) and Gwaii Haanas (BC). The project used risk assessments of invasive plants previously conducted as part of the CFIA's plant protection program, which were then screened based on knowledge of the national parks' climate, geography, and environmental conditions to determine which species posed a potential threat to each park. Park staff will use these preliminary lists as a starting point, to focus early detection and surveillance activities on these species and continue to add more species in the future. The CFIA and Parks Canada have recently signed a memorandum of understanding related to plant health surveillance and research activities which will continue to promote national collaboration in the protection of park environments, and improve information sharing and research synergy.

SOURCE: Frey, M. (2017) An invasive plant watch list for the national capital regional national parks (USA). Natural Areas Journal, 37(1):108-117.

11 Hardy kiwi spreading in New England - new crop or emerging pest?

Actinidia arguta (hardy kiwi) is a vigorous twining woody vine native to regions of eastern temperate Asia. First introduced into North America in 1876 from Japan, by the 1890s it had become a popular ornamental plant in New England, especially in estate gardens. Most of these original plants were likely selected for their vegetative characteristics. As an ornamental, this species is of interest due to its tremendous growth potential, adaptability to various soil types and tolerance of full sun to partial shade. It is also of interest as a source of edible fruit. In its native range (e.g., northeast China), fruit crops are wild-harvested annually and there has been commercial interest in developing hardy kiwi as an alternative to the more familiar sub-tropical kiwifruit (*Actinidia deliciosa*). Since the 1990s, a few small commercial orchards have been established in North America and fruit can be found seasonally, to a limited extent, in both the U.S. and Canada. Furthermore, nurseries have recently begun offering varieties selected for fruiting characteristics to home gardeners.

However, unmanaged populations have been found in several northeastern states, including New Jersey, New York and Massachusetts. This species has also been recently added to the Floras of Maine, Ohio, Pennsylvania and Connecticut. Populations are often found within and along forests margins, abandoned homesteads, roadsides and other disturbed locations. In heavily infested forest canopies, the increased weight from snow and ice accumulation on the vines causes trees to break and fall, creating gaps and allowing this species to dominate sub-canopy layers. Vines lying on soil may root, allowing for clonal expansion and limiting native species regeneration. There is concern that the recent interest in varieties selected for fruiting characteristics is leading to its naturalization in New England. However, the provenance of these unmanaged populations is not entirely clear. Currently, there is much uncertainty as to whether these unmanaged populations are the relics of historical ornamental plantings or if

they are the result of more recent establishment and spread from the varieties selected for fruit characteristics.

Evidently, *Actinidia arguta* is gaining recognition as both a new crop and a new pest in the U.S. Despite its possibility as an alternative kiwi for cooler climates, its potential ability to establish and spread outside of cultivation suggests that it is a species worth monitoring in the future. The state of Massachusetts, for instance, is considering the addition of this species to their prohibited plant list. In light of *Actinidia arguta* being cultivated to a limited extent in Canada, and considering the uncertainty about the naturalized populations in the northeastern U.S., it may be a species of potential concern to Canada as well. It is estimated to be hardy to USDA plant hardiness zone 5, which in Canada includes significant areas of British Columbia, southern Ontario and Quebec as well as the Atlantic provinces.

SOURCES: Demcha, K. (2013) Hardy Kiwifruit: Invasive Plant? Or Throwback to the Gilded Age? Penn State Extension, Tree Fruit Production news publication. [Online] Available: http://extension.psu.edu/plants/tree-fruit/news/2013/hardy-kiwifruit-invasive-plant-or-throwback-to-the-gilded-age

Hale, I. L. and Connolly, B. A. (2014) *Actinidia arguta* (Actinidiaceae): A new record of a naturalized introduction in Connecticut. Rhodora 116(967): 352-355.

Lamont, E. E., Werier, D. and Glenn, S. D. (2014) Noteworthy plants reported from the Torrey Range - 2011 and 2012. The Journal of the Torrey Botanical Society 141(1):95-108.

MDAR (2017a) Massachusetts Department of Agricultural Resources Notice of Public Hearing pursuant to M.G.L.C. 128 Section 27. Boston, MA. [Online] Available: http://www.mass.gov/eea/docs/agr/docs/notice-of-hearing-prohibited-plant-list.pdf

MDAR (2017b) Massachussets Department of Agricutural Resources – Addition of plants onto the Massachusetts Prohibited Plant List department letter. Bostaon, MA. [Online] Available: http://www.mass.gov/eea/docs/agr/farmproducts/docs/prohibited-plant-list-notice-feb2017.pdf

Miller, G. (2015) Hardy kiwi removal in Kennedy Park. Division of Fish and Wildlife (Natural Heritage and Endangered Species Program, Mass Audubon and the Town of Lennox, Boston, MA. [Online] Availble: http://www.mass.gov/eea/agencies/dfg/dfw/wildlife-habitat-conservation/miller-case-studies-for-successful-management-on-town-and-land-trust-lands.pdf

Biotechnology

12 Potential Impact of Novel β-pore-forming Proteins on Future Safety Assessments

Most insect resistant crops derived from biotechnology demonstrate traits based on *Bacillus thuringiensis* insecticidal proteins. 3-domain crystalline (Cry) proteins and vegetative insecticidal proteins (VIP's) encompass the majority of insect resistance traits currently introduced in crops. Recently, there has been increased interest in commercializing novel non-3-domain insecticidal proteins. Novel non-3-domain proteins and their safety was the topic of symposia in 2014 and 2015 that are summarized in a recent Journal of Invertebrate Pathology Special Issue (Moar et al., 2017). The goal of the symposia was to review current information on the structure and function of new insecticidal proteins and discuss how it can be utilized to address topics required for regulatory approval, with an emphasis on target specificity and product safety. The Special Issue explores known and novel protein structures and discusses how these structures impact species specificity and safety.

Recent focus has been placed on the β-pore-forming protein (β-PFP) class. β-PFP's are structurally diverse, and are generally secreted by bacterium as soluble proteins that bind via receptors, and create pores in the membrane of the targeted cells. β-PFP differ from the 3-domain

proteins based on their tail domain structure; 3-domain proteins have a tail domain consisting of β-helices while β-PFP's utilize β-hairpins. The use of whole protein bioinformatics and biochemical analyses has allowed researchers to elucidate the structures of these proteins. These proteins share some similarity at some levels (e.g. Cry35 and Cry51variants share the β-PFP structure with known mammalian toxins such as epsilon toxin) yet have distinct sequences in receptor binding domains (e.g. Cry35 and Cry51variants have differences in their binding domains from epsilon toxins that target pest insect species). Binding domains help determine which cells and species may be affected.

Understanding the implications of protein bioinformatics has introduced a new line of inquiry with regards to regulating these novel proteins. For future safety assessments of novel proteins, this issue proposes a protein safety paradigm based on two tiers. Tier 1 focuses on hazard identification and encompasses protein safety data that informs hazard identification while Tier 2 focuses on hazard characterization in response to concerns identified in Tier 1.

Protein safety data, including bioinformatics, can involve sequence, structure, and functional information to characterize each protein domain individually or as a whole to help inform the tiered approach for hazard identification of the protein safety assessment (Tier 1). This tiered approach is based on the understanding that protein function is a result of the overall structure of a protein with each secondary domain responsible for different/specific aspects of protein function. Each domain may have differential effects on species and cell receptor specificity which can result in different biosafety concerns. If biosafety concerns are raised in Tier 1 (hazard identification), Moar et al. (2017) propose that Tier 2 is implemented to characterize hazards with further testing. With respect to the β-PFP's, some protein domains are mainly involved in forming the pore in targeted cells while other domains have been shown to determine cell receptor binding which confers species specificity. By including protein structure details into the proposed tiered safety assessment paradigm, Moar et al. (2017) propose that our ability to predict the biosafety of these novel insecticidal proteins will increase. The information presented in this special issue indicates we may see a change in the information and safety assessment approaches used by regulated parties interested in commercializing these non 3-domain proteins in the future.

SOURCE: Moar, W. J., Berry, C. and Narva, K. E. (2017). The structure/function of new insecticidal proteins and regulatory challenges for commercialization. Journal of invertebrate pathology 142:1.

13 Aflatoxin free transgenic corn by using RNAi technology

Controlling crop pests and pathogens are major agricultural challenges. While there are a variety of chemical and non-chemical options for growers to use, RNA interference (RNAi) has been shown to be a useful pest management strategy in corn. RNAi works by specifically knocking down essential genes in pests or pathogens, which lessens their fitness and results in their death.

In a previous Science Scan, it was reported that DuPont had developed maize varieties with RNAi targeting western corn rootworm (Hu et al., 2016). More recently, scientists at the University of Arizona reported that RNAi can be used to manage *Aspergillus* infections in corn, and supress the

accumulation of aflatoxins (Thakare et al., 2017).

Aspergillus infection and aflatoxin contamination result in the loss of millions of tons of corn per year, which contributes to world-wide food insecurity. In the US alone, the annual economic damage due to aflatoxin contamination is over a quarter billion dollars. Aflatoxins are potent mycotoxins that seriously damage the liver, promote cancer, cause liver and brain swelling, and certain forms of human dietary protein deficiency.

Because of these economic and health impacts, there have been remarkable efforts to determine the biochemical pathways that yield aflatoxins, and today these pathways are well characterized at both the enzyme and gene levels. This kind of biochemical and molecular knowledge is essential for picking useful RNAi targets in *Aspergillus*. The scientists from the University of Arizona identified a polyketide synthase – *aflC* – because it is unique to *Aspergillus*, did not have any sequence similarity to the corn genome, and a large region of the gene could be engineered into the RNAi construct. It was hypothesized that this last aspect might amplify the anti-fungal and anti-mycotoxin affects, in contrast to smaller, more typical RNAi constructs. Furthermore, in an effort to manage the exposure of non-target organisms to the expressed RNAi molecules, expression was limited to corn kernels – where *Aspergillus* infections are typically localized.

Once transferred to corn, the RNAi was observed to indeed supress the expression of *Aspergillus aflC* polyketide synthase, in comparison to non-RNAi corn lines. Furthermore, aflatoxin accumulation was not detectable in RNAi corn lines, whereas non-RNAi lines had aflatoxin levels between 10 and 14 parts-per-billion. Clearly, RNAi has remarkable applications in managing fungal pathogens of plants, and the accumulation of mycotoxins in agricultural commodities.

As a Plant with Novel Trait (PNT), this RNAi corn variety would have to go through a thorough pre-market safety assessment for unconfined environmental release in Canada.

SOURCES: Thakare, D, Zhang, J, Wing, R, Cotty, P and Schmidt, M. (2017) Aflatoxin-free transgenic maize using host-induced gene silencing. Science Advances 3: DOI: 10.1126/sciadv.1602382

Hu, X, Richtman, N, Zhao, J, Duncan, K., Niu, X., Procyk, L., Oneal, M., Kernodle, B., Steimel, J., Crane, V., Sandahl, G., Ritland, J., Howard, R., Presnail, J., Lu, A. and Wu, G. (2016) Discovery of midgut genes for RNA interference control of corn rootworm. Nature Scientific Reports 6: 30542.

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