PLANT SCIENCE



Edition 14, November 2015

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



- New Host: First record the needle nematode, Longidorus attenuatus, on soybean, Glycine max
- New Technology: Multiplex real-time PCR assay for detection and discrimination of Puccinia horiana and P. chrysanthemi
- 3 New Pest: Population genomics of Dickeya solani, an emerging soft rot bacterium infecting potatoes in Europe



- Survey Tool: Male-produced pheromone component identified for citrus longhorned beetle, Anoplophora chinensis
- Update: Genetic variation in thermal requirements for egg hatch of gypsy moth highlights need for continued vigilance to prevent accidental introduction
- **New Treatment:** A novel quarantine treatment for table grapes using ethyl formate fumigation during cooling

Botany

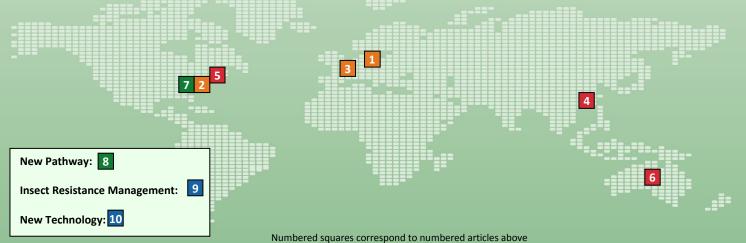
- New Technology: Molecular techniques for distinguishing morphologically similar aquatic plants
- New Pathway: E-commerce as a potential pathway for invasive plant introductions



Insect Resistance Management: Predator-prey relationships might matter

Canada

New Technology: The CRISPR craze





Canadian Food

Inspection Agency



Pathology

1 New Host: First record of the needle nematode, *Longidorus attenuatus*, on soybean, *Glycine max*

In 2014, the needle nematode *Longidorus* attenuatus Hooper was isolated from the rhizosphere of *Glycine max* (soybean) plants in a field in Poland. During greenhouse experiments it was shown that *L. attenuatus* can reproduce on the roots of *G. max* plants; however, no obvious symptoms were observed on the inoculated plants, although these had visibly more yellowing leaves compared to the control plants. This is the first report of *L. attenuatus* associated with *G. max*.

Longidorus attenuatus parasitizes a number of other economically important hosts, including Solanum tuberosum (potato), Triticum sp. (wheat), Beta vulgaris (beet), Fragaria × ananassa (strawberry), Hordeum vulgare (barley), and Daucus carota (carrot). In addition, this nematode is a known vector of the nepovirus Tomato black ring virus (TBRV), a devastating pathogen of grape. Longidorus attenuatus has been reported from Africa and Europe, but is not known to occur in Canada or the United States.

The genus *Longidorus* is currently regulated in Canada on grapevine (*Vitis* sp.) propagative materials, as these nematodes are vectors of important grapevine viruses. *Longidorus attenuatus* can be spread through the movement of infested soil, poorly sanitized bare-rooted plants or contaminated machinery.

SOURCE: Kornobis, F.W., R. Dobosz, P. Bubniewicz, and A. Filipiak. **2015**. First record of nematode *Longidorus attenuatus* on soybean in Poland. Plant Disease doi: 10.1094/PDIS-06-15-0625-PDN.

2 New Technology: Multiplex real-time PCR assay for detection and discrimination of *Puccinia horiana* and *P. chrysanthemi* on chrysanthemum

Puccinia horiana, the cause of chrysanthemum white rust (CWR), is a serious fungal disease of common chrysanthemums grown within the floriculture industry. Symptoms include chlorotic spots on the upper surface of leaves, pustule formation on lower leaf surfaces and defoliation. The disease is spread by infected host material in which it can remain systemic, but not visible, facilitating rapid spread within greenhouses and frequently causing complete crop loss. Another common rust fungi on chrysanthemum, P. chrysanthemi, causes brown rust which damages the cosmetic value of infected plants, but less often causes serious yield loss and is therefore of less concern to the industry.

Although the two species can be distinguished by spore morphology, visual identification is not possible in the latent stages without mature spores or symptoms. Conventional and real-time PCRbased detection assays have been developed for P. horiana in the past, however a recent study out of the USDA-ARS Systematic Mycology and Microbiology Laboratory describes the development of a multiplex real-time PCR assay that simultaneously detects and discriminates P. horiana and P. chrysanthemi. Both species were accurately detected from all fresh samples tested, with as little as 1 pg of template DNA. This multiplex assay could be used for detecting plants infected with P. horiana to ensure that appropriate quarantine measures are applied and that stock plants are free of the pathogen.

Puccinia horiana is a regulated plant pathogen in several countries, including Canada and the United

States. Under the Canada-United States Regulatory Council (RCC) Initiative, the CFIA and USDA are working together on a pilot project that focuses on the coordination of approaches to regulatory oversight for CWR. In 2013-2014, the USDA put forward four proposed regulatory options for *P. horiana* for public comment. These comments are currently under review.

SOURCE: Demers, J.E., Crouch, J.A. and Castlebury, L.A. 2015. A multiplex real-time PCR assay for the detection of *Puccinia horiana* and *P. chrysanthemi* on chrysanthemum. Plant Disease 99: 195-200.

3 New Pest: Population genomics of Dickeya solani, an emerging soft rot bacterium infecting potatoes in Europe

Dickeya species are bacteria that cause soft rot and blackleg diseases in a range of plants and crops worldwide. Formerly Erwinia chrysanthemi, the genus was divided into seven species, namely D. dianthicola, D. dadantii, D. zeae, D. chrysanthemi, D. paradisiacal, D. dieffenbachiae and D. solani (Czajkowski et al. 2013, Samson et al. 2005, Toth et al. 2011). All species have been detected in potato; however, D. dianthicola and D. solani are the only species that have spread to potato in Europe.

Dickeya sp. was first reported on potato in the Netherlands in 1972. These earlier isolates were later classified as *D. dianthicola*, a species that has since spread to several European countries. For years, the species was confined to warm climates and not a significant problem on potato in cool climatic conditions. In 2004, *Dickeya* strains were detected in Finland and have since spread across Europe via trade in seed tubers (Toth et al. 2011). These strains were identified as a new clade belonging to biovar 3, and officially described recently as *D. solani* (van der Wolf et al. 2014). Since its emergence, this new highly aggressive species has been responsible for significant potato

crop losses in Europe due to a higher degree of pathogenicity and symptom severity (Toth et al. 2011).

A recent study by Khayi et al. (2015) provides insight into the genetic traits that may support the aggressive nature of *D. solani*. Using a population genomics approach, the authors analyzed the whole genome polymorphism of 20 D. solani isolates, including the type strain IPO2222^T, collected from different geographical locations, dates of isolation and plant hosts. Illumina and PacBio technologies were used to complete the 3337 D. solani strain genome and which was used as a reference for the comparative genomics. This revealed an unexpected variability among D. solani isolates resulting from a combination of scattered SNP/InDel variations as well as replacing and additive horizontal gene transfer (HGT) events. While most strains belonged to a core population that exhibited less than one hundred variant positions between two given genomes, some other genomes revealed massive replacing HGT from the companion pathogen *D. dianthicola* and a plasmid acquisition. Furthermore, the authors were able to correlate SNPs in flagellar genes with a decrease in motility and virulence, highlighting the power of genomics as a tool to reveal functional variability. Although D. solani has been described as a homogenous population, the results of this analysis encourage the use of multiple taxonomic markers for molecular diagnosis of this emerging pathogen.

Dickeya solani is not currently a regulated pest in Canada; however, the increasing economic losses for potato production in Europe, including cooler Northern regions, signify that it could be a pest of potential quarantine significance.

SOURCES: Czajkowski R., de Boer W.J., van der Zouwen P.S., Kastelein P., Jafra S., de Haan E.G., van den Bovenkamp G.W. and van der Wolf J.M. 2013. Virulence of 'Dickeya solani' and Dickeya dianthicola biovar-1 and -7 strains on potato (Solanum tuberosum). Plant Pathology: 62(3) 597–610.

Khayi, S., Blin, P., Pédron, J., Chong, T-K., Chan, K-G. Moumni, M., Hélias, V. Van Gijsegem, F. and Faure, D. 2015. Population genomics reveals additive and replacing horizontal gene transfers in the emerging pathogen *Dickeya solani*. BMC Genomic 16: 788.

Samson R., Legendre J.B., Christen R., Fischer-Le S.M, Achouak W. and Gardan L. 2005. Transfer of *Pectobacterium chrysanthemi* (Burkholder et al., 1953) Brenner I. 1973 and *Brenneria paradisiaca* to the genus Dickeya gen. nov. as *Dickeya chrysanthemi* comb. nov and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. Internation Journal of Systematic and Evolutionary Microbiology 55: 1415–1427.

Toth, I.K., van der Wolf, J.M., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., Tsror, L. and Elphinstone, J.G. 2011. *Dickeya* species: an emerging problem for potato production in Europe. Plant Pathology 60: 385–399.

van der Wolf, J.M., Nijhuis, E.H., Kowalewska, M.J., Saddler, G.S., Parkinson, N., Elphinstone, J.G., et al. 2014. *Dickeya solani* sp. nov., a pectinolytic plant-pathogenic bacterium isolated from potato (*Solanum tuberosum*). International Journal of Systematic and Evolutionary Micriobiology 64: 768–774.



Entomology

4 Survey Tool: Male-produced pheromone component identified for citrus longhorned beetle, *Anoplophora chinensis*

Long-range pheromones are frequently conserved among closely related species of cerambycids, making semiochemical-bated trapping a method that has the potential to greatly improve the sensitivity and efficiency of detecting populations of these invasive longhorned beetles. Earlier research suggests that the pheromone of the Asian longhorned beetle, *Anoplophora glabripennis*, could have the same or very similar hydroxyether structure to those of the closely related citrus longhorned beetle, *A. chinensis*. This hypothesis was supported by preliminary field bioassays

conducted in China and confirmed in a recent study which evaluated the response of *A. chinensis* to 4-(n-heptyloxy)butan-1-ol, a component of the volatile pheromone produced by *A. glabripennis*.

Headspace volatiles were collected from beetles of both sexes held in aeration chambers and screened by coupled gas chromatography-mass spectrometry. Both 4-(n-heptyloxy)butan-1-ol (alcohol) and 4-(n-heptyloxy)butanal (aldehyde) were detected in headspace volatiles from male A. chinensis, but not in volatiles from females. Coupled gas chromatography-electroantennogram analyses demonstrated that the antennae of both males and females responded strongly to the alcohol, but not the aldehyde. Field trials were performed using pheromone lures baited with these compounds, which confirmed that both sexes were significantly attracted to traps baited with the alcohol alone or blended 1:1 with the aldehyde.

This study provides evidence that 4-(n-heptyloxy)butan-1-ol is an important component of the volatile pheromone produced by male A. chinensis and the fact that beetles of both sexes were attracted indicates that the alcohol is an aggregation pheromone, even though its primary function is likely to bring sexes together for mating. The results suggest that the alcohol would be an excellent candidate for a lure to detect new invasions of A. chinensis in North America and other parts of the world.

Longohorned beetles, *Anoplophora* spp., are regulated pests in Canada. *Anoplophora chinensis* is a polyphagous wood-boring beetle that is able to infest and kill living trees of a broad host range. It is native to East Asia, but has also become established in several European countries where it is predicted to cause environmental and economic damage. It has also been detected at least twice in

North America, in Georgia and Washington, but appears to not have become established.

SOURCE: Hansen, L., Xu, T., Wickham, J., Chen Y, Hao, D. Hanks, L.M. et al. 2015. Identification of a Male-Produced Pheromone Component of the Citrus Longhorned Beetle, *Anoplophora chinensis*. PLoS ONE 10(8): e0134358.

5 Update: Genetic variation in thermal requirements for egg hatch of gypsy moth highlights need for continued vigilance to prevent accidental introduction

Gypsy moth, Lymantria dispar, is a univoltine species throughout its range that spends eight to nine months per year in diapause as a mature larva within the egg. Once diapause requirements have been satisfied, the egg becomes responsive to spring temperatures allowing hatch to synchronize with bud-burst of hosts. Differences in timing and percentage of egg hatch after being exposed to different temperatures and durations of low temperature have been documented for populations from the Russian Far East and the United States (Keena 1996). Eggs from the Russian populations required less exposure to low temperature to satisfy diapause requirements which allowed them to complete post-diapause more quickly and hatch when moved to warmer temperatures. There is a concern that Asian gypsy moth populations may all possess this reduced requirement for egg chill to break diapause which could increase the risk of introduction and potential establishment.

In a recent paper, Keen (2015) assessed mode of inheritance of hatch traits in *L. dispar* by crossing populations nearly fixed for the phenotypic extremes including a North American population with almost no egg hatch after being chilled for 60 days at 5°C, a Russian population of which >80% of eggs hatched after being exposed to the same

conditions and a Russian population selected for non-diapause. Hatch traits, such as days to first hatch, duration of hatch and percentage hatch, were also evaluated for eggs from 43 geographic populations, including locations in Europe, China, Japan, Russia and the United States.

Results indicated that the non-diapausing phenotype was inherited via a single recessive gene and the phenotype with reduced low temperature exposure requirements before hatch was inherited via a single dominant gene. Hatch traits varied considerably both within and among populations when eggs from the 43 populations were chilled at 5°C then incubated at 25°C. This is likely an adaptation to local climate and variation within a population would provide a strategy to ensure that at least some hatch synchronizes with host leaf-out. These results highlight the need for continued vigilance to prevent movement of populations both within and between countries. Alleles that confer non-diapause or reduced low temperature requirements are not present in all populations and therefore their introduction into a new area could increase variation of egg hatch traits within a population and the ability to adapt to environmental uncertainty. The ability to forecast egg hatch is also critical to time control programs and predict the ability of these populations to adapt to the range of climates found in North America.

SOURCES: Keena, M.A. 1996. Comparison of hatch of *Lymantria dispar* (Lepidoptera: Lymantriidae) eggs from Russia and the United States after exposure to different temperatures and durations of low temperature. Annals of the Entomological Society of America 89: 564-572.

Keena, M. A. 2015. Inheritance and World Variation in Thermal Requirements for Egg Hatch in *Lymantria dispar* (Lepidoptera: Erebidae). Environmental Entomology 1-10 (Advance Access), doi: 10.1093/ee/nvv163.

6 New Treatment: A novel quarantine treatment for table grapes using ethyl formate fumigation

Ethyl formate (EF) is a plant volatile that has been shown to have insecticidal properties. It occurs naturally in a variety of products, including essential oils of grasses, beer, rice, beef and cheese, as well as volatile components of grapes and wine. One important advantage of using volatiles such as EF for fumigation is that residues found on treated commodities are found only in trace amounts (Simpson et al. 2007).

In a recent study from the Department of Agriculture and Food Western Australia, De Lima (2015) investigated the effectiveness of EF in combination with low temperatures on table grapes. Grapes were harvested directly into ventilated polystyrene boxes and chilled to approximately 20°C for six to eight hours before applying 52.6 g m⁻³ EF + 21.6% CO₂ to treat external pests and stages likely to be found in harvested produce from Western Australia: 1st-3rd instar light brown apple moth and red back spiders, longtailed mealy bug crawlers, adult two spotted spider mites, plague thrips and western flower thrips. Fumigation was applied for 2.5 hours in a refrigerated shipping container while cooling to 15°C. Cooling continued to 1 and 2°C for 16 and 18 days, respectively to simulate the cold disinfestation stage that is applied for control of the Mediterranean and Queensland fruit flies. The results showed that all species were killed through this fumigation process and that the optimum quality of grapes was still maintained.

This study provides new information on quarantine application of EF+CO₂ and cold disinfestation for market access of Australian table grapes. This novel treatment could be applied for certain regulated

pests in Canada, such as light brown apple moth, *Epiphyas postvittana*, which is currently regulated on grapes from Australia.

SOURCES: De Lima C. P. F. 2015. Maintaining Table Grape Quality by Applying Quarantine Treatments during Cooling. Acta Horticulturae 1091: 55-61.

Simpson, T., Bikoba, V., Tipping, C. and Mitcham, E.J. 2007. Ethyl Formate as a Postharvest Fumigant for Selected Pests of Table Grapes. Journal of Economic Entomology 100(4): 1084-1090.



Botany

7 New Technology: Molecular techniques for distinguishing morphologically similar aquatic plants

A new study has applied molecular techniques to distinguish four aquatic plant species that are morphologically similar and difficult to tell apart: Hydrilla (Hydrilla verticillata (L. f. Royle), Brazillian waterweed (Egeria densa Planch.), Canada waterweed (Elodea canadensis Michx.) and Nuttall's waterweed (Elodea nuttallii (Planch.) H. St. John). All four are submerged macrophytes of the frogbit family (Hydrocharitaceae) that are invasive and problematic on several continents. They reproduce vegetatively via stem fragmentation and have non-dissected leaves occurring in whorls, making them look very similar. Hydrilla and Brazilian waterweed are considered invasive in North America, while Canada waterweed and Nuttall's waterweed are native in North America but invasive in Europe.

The authors used existing molecular techniques to develop a simple protocol, using DNA sequenced samples as a standard for each species, and then tested and confirmed the protocol on reference samples and herbarium and live specimens

collected from across the U.S. They used a polymerase chain reaction (PCR) assay and previously developed primer pair (ITS5³ and 26S25R³, targeting the nuclear rRNA ITS region ITS1, 5.8srRNA, ITS2) to distinguish hydrilla from the waterweeds by amplified length heterogeneity (ALH), and additional steps using restriction fragment length polymorphism (RFLP) analysis to distinguish Brazilian from the two native waterweeds and the two native waterweeds from each other. Of the 105 samples collected (including reference and standard samples), 83 yielded amplifiable, uncontaminated DNA, with the best results coming from plants that were frozen fresh or freeze dried so that green colour was retained. Older samples that were dried and lacked any green pigment were most likely to have degraded DNA. The age of the successfully identified samples ranged from fresh to about 40 years old. The time needed to differentiate hydrilla was about 4 hours, with additional RFLP steps requiring an additional day to complete.

The results of this study have improved the understanding of past and present distributions of these species in North America. A number of misidentified specimens have been corrected, and recorded distributions adjusted accordingly. The distribution of Nuttall's waterweed is wider than previously thought, while that of Canada waterweed is narrower. Hydrilla arrived in both the Potomac River and Chesapeake Bay earlier than previously thought, revising earlier ideas of how it was introduced into the area. Both biotypes of hydrilla are shown to be spreading northwards, towards the Canadian border. Samples from stores in the U.S. indicate that Brazilian waterweed continues to be sold despite regulatory restrictions in a number of states, and one sample of hydrilla from an ornamental pond where it was not

knowingly planted illustrates the unintentional transport of invasive species in the aquarium and water garden trade. The authors particularly note the importance of positive identification in the success of aquatic plant management plans. Quick and accurate identification is needed for early detection, targeted control efforts, and prevention of further spread. Molecular techniques such as these could be useful in distinguishing alien invasive species such as hydrilla and Brazilian waterweed from native species, if they are regulated or controlled in Canada in the future.

SOURCES: Rybicki, N. B., Kirshtein, J. B. and Voytek, M. A. 2013. Molecular techniques to distinguish morphologically similar *Hydrilla verticillata*, *Egeria densa*, *Elodea nuttallii* and *Elodea canadensis*. Journal of Aquatic Plant Management 51: 94-102.

USGS 2014. Technical Announcement: New DNA tool helps scientists identify invasive species of aquatic plants. United States Department of the Interior / United States Geological Survey (USGS), Office of Communications and Publishing, Reston, VA. [Online] Available: http://www.usgs.gov/newsroom/article.asp?ID=3860#.VWeFFLGVIL M [Accessed 2015].

8 New Pathway: E-commerce as a potential pathway for invasive plant introductions

A recent study in *Conservation Biology* investigated e-commerce as a potential pathway for invasive plant introductions, and proposed measures for increasing biosecurity through the identification of invasive species online before they become widely distributed.

The authors developed software that automated search algorithms and downloaded information on all plant offers from eBay.com based on a predefined list of plant species (Humair et al. 2015). eBay.com was selected as it is one of the world's largest online marketplaces, and the pre-defined list of plant species was compiled from the website Global Flora and the Invasive Species List. Searches

were conducted over a period of 50 days between February and April 2014, and the location of online species was determined using information from the item location field of each offer.

The study found over 2600 plant species for sale, with over 500 of the species being invasive. Of the 100 most frequently offered plant species, 41 were invasive, with 13 being listed on the IUCN's World's Worst Invasive Alien species list (IUCN 2014). Plants were available from numerous countries and world regions, and most vendors offered to ship plants worldwide.

Many plant species recognized as harmful and invasive are currently being offered daily across the internet from around the globe. Invasive species were even for sale in countries that are considered leaders in invasive species prevention, such as Australia, the United States, and Europe. This study highlights a gap in preventative phytosanitary measures and the need to strengthen online biosecurity measures. The automated surveillance of select online auctions could be relatively simple to implement, but developing a system that completely captures the range of online plant trade may be more difficult, and could require coordination between national and regional (e.g. EU) regulatory bodies. The CFIA's Invasive Alien Species and Domestic Program Section has recognized e-commerce as a priority and is currently working on the issue with the U.S., Australia and New Zealand.

SOURCES: Humair, F., Humair, L., Kuhn, F., and Kueffer, C. 2015. Ecommerce trade in invasive plants. Conservation Biology. Volume 00(0): 1-8.

IUCN (International Union for Conservation of Nature). 2014. 100 of the world's worst invasive alien species. [Online] Available: http://www.issg.org/database/species/search.asp?st=100ss [Accessed September 2015].



Biotechnology

9 Insect resistance management: predator-prey relationships might matter

Insect resistance management (IRM) plans are mandated by the CFIA for some plants with novel traits that confer resistance to insect feeding, such as Bt corn. Current IRM plans are largely based on planting a refuge of an unmodified crop to maintain a population of susceptible insects and delay the development of populations of resistant insects. As more is learned about IRM practices, further consideration is being given to how complex factors may influence the evolution of resistance in target pests. An example of this is how predators may interact with both prey species and crops to delay or accelerate the evolution of resistance. If predators selectively prey on resistant individuals, they may slow the evolution of resistance in a population. Alternatively, if predators select susceptible individuals, they may accelerate the evolution of resistance.

A study by Liu et al. (2015) evaluated the effect of prey genotypes (susceptible or resistant) and their interaction with plant type on a predator over two generations. The test system included broccoli expressing the Bt protein Cry1Ac, the diamondback moth *Plutella xylostella* (a pest controlled by Cry1Ac), and the spotted lady beetle *Coleomegilla maculata* (a predator of diamondback moth larvae). The spotted lady beetle did not distinguish between Bt and non-Bt plant types and did not prefer resistant or susceptible diamondback moth larvae. Thus, it is not expected that the spotted lady beetle would directly alter the evolution of resistance in diamondback moth populations; however, similar greenhouse studies found that in

combination with a refuge the spotted lady beetle may slow the evolution of resistance. Field studies in a variety of systems are required to further evaluate the potential implications of predatorprey relationships on IRM.

The spotted lady beetle was not affected by Cry1Ac-fed prey; this supports the safety of Bt crops for natural enemies. Bt crops and biological control may be used synergistically within an integrated pest management program to control secondary pests and reduce insecticide use. It is possible that predators may be manipulated in some systems to delay the evolution of pest resistance to plants with novel traits.

SOURCE: Liu, X., Abro, G.H., Han, F., Tian, J., Chen, M., Onstad, D., Roush, R., Zhang, Q., and Shelton, A.M. 2015. Effect of Bt broccoli and resistant genotype of *Plutella xylostella* (Lepidoptera: Plutellidae) on life history and prey acceptance of the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). Biological Control 91: 55-61.

10 New Technology: The CRISPR craze

In nature, clustered regularly interspaced palindromic repeat (CRISPR) arrays confer adaptive, pathogen immunity to bacteria. This form of immunity has been redeveloped for biotech purposes and lends itself well to editing genomic sequences. Since 2013, genome editing using CRISPR-Cas9 tools has been reported for ten plants; notable crops are rice, sorghum, tomato, tobacco, wheat, maize and sweet orange. The hopes are that the lowered costs of CRISPR-Cas9 tools, in comparison to existing sequence specific nucleases (SSN), and the democratization of these tools will lead to improving crops like cassava, which are important in the developing world. The recent explosion of interest in CRISPR-Cas9 tools can be traced to its features; Firstly, CRISPR-Cas9 SSN target DNA using a guide RNA (gRNA) and Watson-Crick pairing rules. By comparison, other

SSNs, like zink finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN), use protein-based DNA interactions which are more costly and difficult to engineer. Secondly, no cloning is required for CRISPR-Cas9 constructs, and multiple genomic loci can be targeted in a single experiment. These two features dramatically improve the throughput of genome editing programs over other SSNs. Furthermore, CRISPR-Cas9 tools can be used to perform targeted insertion-deletion mutations, as well as introduce exogenous DNA from distantly related organisms. Lastly, in contrast to the proprietary nature of ZFNs, the CRISPR-Cas9 technology is open-access. Resources like plasmid requisition, gRNA specificity prediction tools and support and discussion forums are available online. These features make the CRISPR-Cas9 system dramatically less expensive to execute and can be more easily developed in new labs when compared to other SSNs.

In terms of applications, one might imagine scientists producing, in a single generation, a low cyanogenic glycoside cassava plant by disrupting every gene involved in cyanogenic glycoside biosynthesis. This is theoretically possible with CRISPR-Cas9 tools. This cassava product would have improved nutritional value and reduced postharvest costs.

It remains to be seen how products of CRISPR-Cas9 tools will be treated by worldwide regulatory agencies. Where these tools are used for targeted mutagenesis purposes, the genome alterations are indistinguishable from those that naturally occur during plant breeding, and this plant product might be regulated as chemically mutated plant varieties are. Where exogenous DNA sequences are introduced, one might imagine that those plant products will be treated similarly to other products of recombinant DNA technologies. Because the

Canadian regulatory system is product based, we are well positioned to address plants generated by this remarkable technology.

SOURCE: Catarino, R., Ceddia, G., Areal, F.J. and Park, J. 2015. The impact of secondary pests on *Bacillus thuringiensis* (Bt) crops. Plant Biotechnology. J., doi: 10.1111/pbi.12363

Acknowledgments

Thanks to the following CFIA staff who contributed to this edition of the Plant Science Scan: A. Ameen, B. Day, S. Gulden, A. Hitchon, W. Laviolette, D. Levac, S. Li, M. Mander, A. Sissons, and C. Wilson.

DISCLAIMER: The Plant Science Scan report is an alert service prepared by the Canadian Food Inspection Agency's staff for personal and non-commercial public use. The views and opinions of authors expressed herein or contained in the articles referred to herein are those of the authors, do not necessarily state or reflect those of the Canadian Food Inspection Agency. Neither the Canadian Food Inspection Agency nor its employees make any representation or warranty, express or implied, of any kind whatsoever, and assume no legal liability or responsibility for the accuracy, reliability, completeness or usefulness of any information, product, process or material supplied by external sources as disclosed by or in this Plant Science Scan report.

All and any reliance on or use of any information, product, process or material supplied by external sources as disclosed by or in this Plant Science Scan report is at the sole risk of the person(s) so using it or relying thereon. Readers should at all times verify any such information, product, process or material and consult directly with the author(s) or source of that information, product, process or material, especially before acting on it or relying upon it for any purposes.

Reference in the Plant Science Scan report to any specific product, process or service, by trade name, trade-mark, manufacturer or otherwise does not necessarily constitute or imply its endorsement or recommendation by the Canadian Food Inspection Agency.

COPYRIGHT / PERMISSION TO REPRODUCE: This Plant Science Scan report and any information, product, process or material supplied by external sources as disclosed by or in this Plant Science Scan report are covered by the provisions of the Copyright Act, by Canadian laws, policies, regulations and international agreements. Such provisions serve to identify the information source and, in specific instances, to prohibit reproduction of materials without written permission. This is particularly true for the reproduction of materials supplied by external sources as disclosed by or in this Plant Science Scan report, as some restrictions may apply; it may be necessary for the users to seek permission from the rights holder prior to reproducing the material.

Non-commercial Reproduction: This Plant Science Scan report has been distributed with the intent that it be readily available for personal and non-commercial public use and may be reproduced, in part or in whole and by any means, without charge or further permission from the Canadian Food Inspection Agency. We ask only that:

- Users exercise due diligence in ensuring the accuracy of the materials reproduced;
- The Canadian Food Inspection Agency be identified as the source department-agency; and,
- The reproduction is not represented as an official version of the materials reproduced, nor as having been made, in affiliation with or with the endorsement of the Canadian Food Inspection Agency.

Commercial Reproduction: Reproduction of multiple copies of this Plant Science Scan report, in whole or in part, for the purposes of commercial redistribution is prohibited except with written permission from the Canadian Food Inspection Agency. To obtain permission to reproduce this Plant Science Scan report for commercial purposes please contact:

Canadian Food Inspection Agency
Plant Science Scan
Tower 1, Floor 1, 1400 Merivale Road
Ottawa, ON, Canada K1A 0Y9
PSS-SSV@inspection.qc.ca