

Edition 4, March 2013

**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.

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### **Pathology**

# 1 Update: Tomato as a latent carrier of 'Candidatus Liberibacter solanacearum', the causal agent of potato zebra chip disease

A recent study conducted at the CFIA examined graft transmission of 'Candidatus Liberibacter solanacearum', the causal agent of potato zebra chip (ZC) disease, an emerging threat to potato. ZC disease occurs in North America from Guatemala to the northern US, New Zealand, and northern Europe. Vectors for the disease include psyllids Bactericera cockerelli and Trioza apicalis. In this study, ZC-infected tomato and potato were produced through graft-inoculation from latently infected tomato. Tomato plants (varieties Moneymaker and Roma) were found to be potential latent carriers of 'Ca. L. solanacearum', and the pathogen was systematically present in both tomato and potato plants. Potato plants (cultivars Jemseg, Atlantic, Shepody, Frontier Russet, and Russet Norkotah) demonstrated symptoms of purple top, leaf scorch, and other disease symptoms, and tested positive for the pathogen in a PCR assay. The potato tubers generated showed typical ZC symptoms of brown discoloration in the vascular ring and medullary rays.

In a second part of this study, a culture-independent approach was employed to differentiate the causal agents of ZC from the potato witches' broom phytoplasm. Both ZC pathogen and witches' broom phytoplasma are ploem limited fastidious bacteria in potato. Using phylogenetics and barcoding they found ample differential sequence targets to differentiate the two pathogens.

ZC disease is not known to occur in Canada nor is it

a regulated pest for Canada. 'Candidatus Liberibacter solanacearum' (Solanaceae haplotypes) and its vector Bactericera cockerelli have recently been added to the EPPO A1 list of pests recommended for regulation as quarantine pests.

#### SOURCES:

QBOL-EPPO Conference on DNA barcoding and diagnostic methods for plants. 2012.

http://archives.eppo.int/MEETINGS/2012 conferences/diagnostic/0 6 Xiang Li/index.html

EPPO Alert List. 2013.

http://www.eppo.int/QUARANTINE/Alert List/bacteria/Liberibacter\_psyllaurous.htm

# 2 Diagnostics: New section on validation data added to EPPO database

Recently, the EPPO Database on Diagnostic Expertise added a new section "Validation data for diagnostic tests". The data has been generated by various laboratories in EPPO member countries for diagnostic testing of regulated pests. Various testing methods are posted included molecular, serological and morphological tests.

Testing data is available for various pest regulated by Canada including, the bois noir phytoplasma, the potato cyst nematodes, the Columbia root-knot nematode, *Phytopthora ramorum*, and plum pox virus.

SOURCE:

EPPO Database on Diagnostic Expertise. 2013. http://dc.eppo.int/validationlist.php

### 3 First report: Potato mop top virus from Idaho

Potato mop-top virus (PMTV) causes an economically important disease of potato since serious yield and quality reductions can occur in some cultivars. Transmission of the virus occurs via the obligate vector, powdery scab fungus (Spongospora subterranea). PMTV tubers planted as seed, can pass the virus on as a secondary

infection to a limited number of progeny tubers. Recently, PMTV was identified in southeastern Idaho from commercially produced potatoes cv. Modoc. All of the potatoes that tested positive for PMTV had internal necrotic arcs and lines.

In Canada PMTV is considered a regulated nonquarantine pest (RNQP) for seed potato certification. Its distribution in Canada is sporadic, but it is more common in coastal areas. PMTV is present in the U.S. including Washington, North Dakota and Maine.

#### SOURCE:

J. L. Whitworth and J. M. Crosslin, 2013. Detection of Potato mop top virus (Furovirus) on potato in southeast Idaho. Plant Disease 97(1):149.

This prioritization process is similar to a categorization process already employed by the CFIA. Categorization is used to quickly evaluate lists of plant species to determine their potential risks to the Canadian economy and environment. The CFIA continues to monitor advancements in weed prioritization and risk assessment around the globe in order to improve its approach to invasive alien plants and harmonize methods where possible.

#### SOURCE:

European and Mediterranean Plant Protection Organization (EPPO). 2012. Guidelines on Pest Risk Analysis – EPPO prioritization process for invasive alien plants. EPPO Bulletin 42(3) 463-474.



### Botany



### **Entomology**

# 4 Update: EPPO prioritization process for invasive alien plants

The European Plant Protection Organization (EPPO) has developed a new standard for prioritizing alien plants. The standard has two main objectives, 1) to develop lists of alien plants for a given area, and 2) to prioritize them for pest risk analysis (PRA). The first objective involves compiling lists of alien plants and filtering them through a simple question-based decision tree into the following categories: 1) minor concern, 2) observation list and 3) invasive alien plants. Questions address establishment, spread and impact. Guidance on rating uncertainty is also provided. The second objective involves assessing list 3 with a second decision tree, to determine each species' priority for PRA. There are three outcomes to this process: 1) not a priority, 2) lower priority and 3) priority. The PRA process is then used to guide recommendations regarding possible international action to prevent introduction and spread.

# 5 Containment: Efficacy of insect containment facilities

The efficacy of an entrance-exit anteroom at Agriculture and AgriFood Canada's Insect Microbial Containment Facility (IMCF) was assessed using insect release-and-recapture methods to test two components of the anteroom: insect traps (either light or moisture), and air flow. The efficacy of traps and air flow varied with insect species, demonstrating the importance of understanding the mobile stages of pests when optimizing containment strategies. Although standard methods were adequate in preventing insect escape, the authors noted that awareness of specific insect behaviours may be required to determine if additional containment measures would be necessary to contain specific quarantine plant pests. This study was conducted to assist in design, operation, use and regulation of insect containment facilities.

In Canada, containment standards are outlined in the CFIA's "Containment standards for facilities handling plant pests" and NAPPO RSPM No. 22. "Guidelines for the construction and operation of a containment facility for insects and mites used as biological control agents".

#### SOURCES:

Canadian Food Inspection Agency (CFIA). 2007. Containment standards for facilities handling plant pests, 1st ed. Catalogue Number A104-65/2008E. Canadian Food Inspection Agency, Ottawa, Canada.

De Clerck-Floate, R.A., Saunders, P. and Floate, K.D. 2012. Release and recapture of three insect species test the efficacy of trap method and air flow in insect containment. The Canadian Entomologist 144(04): 609-616.

North American Plant Protection Organization (NAPPO). 2004. North American Plant Protection Organization regional standard for phytosanitary measures (RSPM) No. 22. Guidelines for the construction and operation of a containment facility for insects and mites used as biological control agents. Secretariat of the North American Plant Protection Organization, Ottawa, Canada.

# 6 New Pest: Polygraphus proximus — a new bark beetle from the East

A recent European Pest Risk Assessment on the bark beetle Polygraphus proximus (Coleoptera: Scolytidae) concluded that should this pest continue to spread it has the potential to establish and cause economic and environmental damage in the whole EPPO region where host plants occur. This species has spread from its native range in eastern Siberia and adjoining countries and has been reported from western Siberia, Moscow province, and Leningrad province. Polygraphus proximus feeds on firs and other conifers; the beetles attack fresh logs and trees, as well as trees weakened by fires, pathogens, or defoliation. It could attack new hosts in the genera Abies (it is known to attack the native North American Abies balsamea in Russian plantations), Pinus, Larix, Picea and Tsuga. Wood with bark of host species was identified as the most likely pathway for entry, and other potential pathways included wood packing material, wood chips, bark, plants for planting and Christmas trees.

Polygraphus proximus is not known to occur in Canada or the U.S. and is not listed as a regulated pest for Canada. Canada regulates the import of non-processed wood and non-propagative wood products to reduce the risk of introduction and spread of pests.

#### SOURCES:

Baranchikov, Y., Akulov, E. and Astapenko, S. 2011. Bark beetle *Polygraphus proximus*: a new aggressive far eastem invader on *Abies* species in Siberia and European Russia. pp 64-65 In: McManus, K.A., Gottschalk, K.W., eds. Proceedings. 21st U.S. Department of Agriculture interagency research forum on invasive species 2010; 2010 January 12-15; Annapolis, MD. Gen. Tech. Rep. NRS-P-75. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

Expert Working Group for performing a PRA on *Polygraphus proximus*. 2012.

http://www.eppo.int/MEETINGS/2012 meetings/ewg polygraphu s.htm? tmc=19qT1KOAUPMeCx2kF7uXWTcrxlt3lik1jz2sYxKJw94

# 7 Phytosanitary Measures: Irradiation effective for Light Brown Apple Moth

The light brown apple moth, Epiphyas postvittana (Lepidoptera: Tortricidae) is a significant economic pest of fruits and nursery plants. It is a pest in its native Australia, and its finding in California in 2007 precipitated quarantine actions that are still ongoing. This study investigated the potential applicability of irradiation as a postharvest quarantine treatment option for light brown apple moth. Currently, the USDA-APHIS has approved irradiation as a viable phytosanitary treatment for certain pests of quarantine significance. This study examined the effects of irradiation on egg, larval, and pupal development, and adult reproduction. The results indicated that tolerance to radiation increased with increasing larval age, and further studies with larger sample sizes are required to determine a radiation treatment level which will provide quarantine security.

The light brown apple moth is a regulated pest in Canada. This pest is not present in Canada, and the CFIA has developed phytosanitary import

requirements to prevent its introduction.

#### SOURCES:

Follett, P.A. and Snook, K. 2012. Irradiation for Quarantine Control of the Invasive Light Brown Apple Moth (Lepidoptera: Tortricidae) and a Generic Dose for Tortricid Eggs and Larvae. Journal of Economic Entomology 105(6): 1971-1978.

NAPPO Phytosanitary Pest Alert System. 2007. http://www.pestalert.org/oprDetail.cfm?oprID=260&keyword=Epiphyas%20postvittana

# 8 Update: Invasion success of Gypsy Moth

Mate-finding is a constraint which may limit the establishment of gypsy moth, Lymantria dispar (Lepidoptera: Lymantriidae) populations. The failure of low density populations to persist is thought to be partly due to mate-finding failures. A recent study compiled data from several previous studies conducted over four decades and evaluated female mating success and its relationship to male density. By examining the proportion of females mated and number of males in traps, the study linked male moth trap catch to expected female mating success. The result was that a density of 4.5 males per trap per day was equated to 50% female mating success. No instances of mated females was found when season-long male moth trap catches were nil. This study shows that male traps can be used to detect new, viable populations and highlights the usefulness of trapping data to inform management. These results can further be used to understand the role of mate-finding failures at low density for other non-native insect pests.

Gypsy moth is a regulated pest in Canada and pheromone-based trapping is routinely used as a means of determining its distribution in Canada.

### SOURCE:

Tobin P.C., Onufrieva, K.S. and Thorpe, K.W. 2013. The relationship between male moth density and female mating success in invading populations of *Lymantria dispar*. Entomologia Experimentalis et Applicata 146(1): 103-111.



### **Biotechnology**

# 9 Update: Consensus document of the biology of the *Brassica* crops (*Brassica* spp.)

The Environment, Health and Safety Division of the Organisation for Economic Cooperation and Development (OECD) recently released the "Consensus document of the Biology of the Brassica Crops (Brassica spp.)". This document was produced as part of an initiative to harmonize regulatory oversight of transgenic plants between OECD member countries thereby increasing the efficiency of safety assessments and promoting transparency. Canada, represented by members of the CFIA, served as the lead country in the preparation of this document. Dr. Keith Downey, a foremost Canadian expert on Brassica species and one of the original developers of canola, was invited to help with the document preparation. Brassica species are among the world's most economically important plants, and this document provides information related to the taxonomy, reproductive biology, genetics, hybridization, ecology, breeding, and common pathogens and pests of *Brassica* species.

The CFIA is responsible for regulating the environmental release of plants with novel traits (PNTs). and baseline information of plant species is essental in order to perform environmental safety assessments of PNTs. As the world's largest single producer of canola (a commercial name for some specific *Brassica* cultivars) the majority of which is genetically modified, Canada has a significant interest in the release of the *Brassica* Consensus document. This document will be a fundmanetal resource for the risk assessment of PNT *Brassica* species for both OECD and non-OECD countries.

#### SOURCE:

Environment, Health and Safety Division of the Organisation for Economic Cooperation and Development (OECD). 2012. Consensus Document on the Biology of the *Brassica* Crops (*Brassica* spp.).

Series on Harmonisation of Regulatory Oversight in Biotechnology No. 54. Paris, France.

### 10 Update: Identification of miRNAs from *Brassica rapa*

MicroRNAs (miRNAs) are short, usually 21 nt, noncoding RNAs involved in the regulation of various biological processes including development. Plant genomes contain both evolutionary conserved miRNAs as well as speciesspecific miRNAs. The ongoing identification and characteization of these molecules is the focus of many studies in plant genomics. A recent study used computational analysis and high throughput sequencing to identify miRNAs in Brassica rapa. The study identified 216 novel and 196 conserved miRNA genes from several tissue types. Bioinformatics was used to predict miRNA targets which included genes involved in diverse molecular function including transporter activity and tranferase activity. Further studies are required to characterize the miRNAs and their interatctions with gene targets.

With the successful sequencing of many plant genomes, including economically important crops, scientitists are now able to study the complex components of the genome. Small RNAs have redefined our understanding of genes control, and genome-wide discovery of miRNA will further our understanding and provide insight into the plant genome.

Studying microRNAs has the potential to impact the CFIA's risk assessments of PNTs as we continue to further our understanding of gene funtion and the possible consequences of disrupting gene areas. Future studies on microRNAs may provide a gateway to novel gene products.

#### SOURCE:

Kim, B., Yu, H., Park, S., Shin, J.Y., Oh, M., Kim, N. and Mun, J. 2012. Identification and profiling of novel microRNAs in the Brassica rapa genome based on small RNA deep sequencing. BMC Plant Biology 12:218.

## 11 New Technology: Transcription activator-like effector nucleases

Engineered restriction enzymes are considered to be one of the most mature technologies in genetic engineering. Artificial nucleases have been used in creating herbicide and disease resistance in plants though gene targeting. Transcription activator-like effector nucleases (TALENs) are site-specific nucleases that can be easily customized and produced to recognize almost any DNA sequence. TALENs are chimeric proteins that are artificially created by fusing engineered transcription activator-like effector (TALE) DNA-binding domains to the catalytic domain of the Fokl endonuclease. Few studies have explored the use of TALENs in plant biotechnology; research by Zhang et. al (2013) used a tobacco protoplast model system in order to optimize TALEN architecture, expression and delivery system. This study looked to achieve efficient and reliable TALEN-based mutagenesis and gene targeting through non-homologous end joining (NHEJ) and homologous recombination respectively. This technology is a refinement on the more traditional, non specific mutagenesis techniques used in plant breeding to introduce new traits of potential interest into plants.

The CFIA is responsible for regulating the environmental release of plants with novel traits (PNTs). The CFIA's regulatory oversight for PNTs is triggered when a plant is developed which contains a trait that is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health.

#### SOURCE:

Zhang, Y., F. Zhang, X. Li, J.A. Baller, Y. Qi, C.G. Starker, A.J. Bogdanove and D.F. Voytas. 2013. Transcription activator-like effector nucleases enable efficient plant genome engineering. Plant Physiology. 161(1):20-7.

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