ISSN 2369-4254

PLANT SCIENCE SCAN

Edition 15, April 2016

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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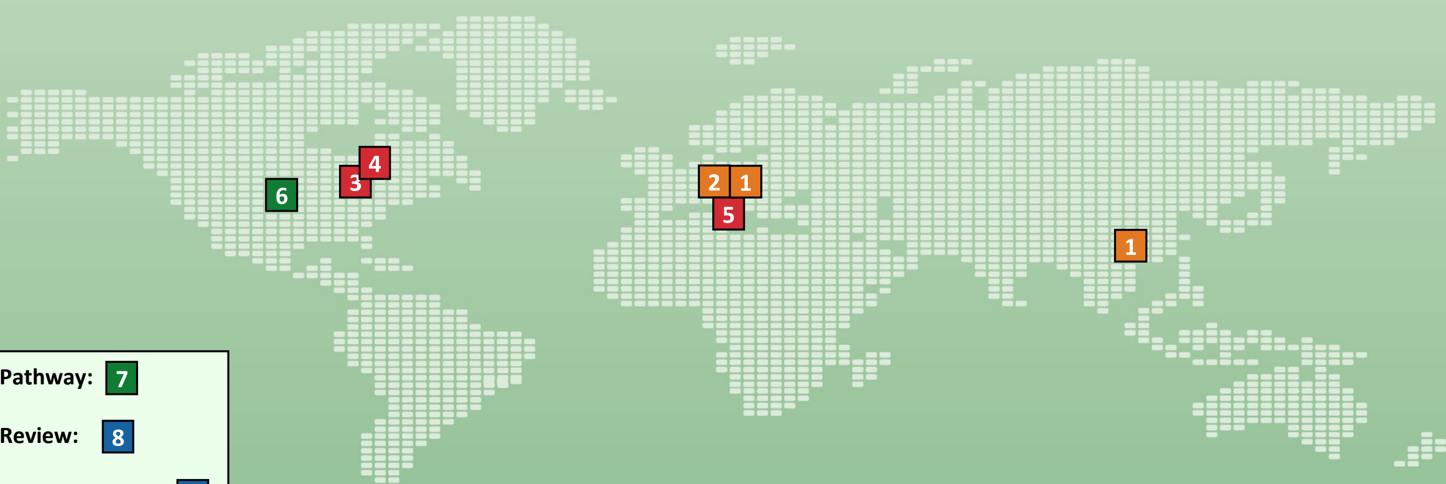

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Pathology

1 New pest: New canker disease on poplar in Europe and Asia

In 2009, a new disease was observed on poplar (*Populus x euramericana*) trees in Hungary. The primary symptom of the disease was vertical cracks in the bark of the tree with a sticky, brown substance oozing from the canker. A bacterium was isolated from the ooze and named *Lonsdalea quercina* subsp. *populi* (Tóth et al., 2013). A paper in the journal *Plant Disease* reports that this bacterium has now also been identified on *Populus x euramericana* in China. Affected trees had symptoms of bark canker with frothy white exudates, and severely affected trees even died (Li et al., 2014).

A closely related species, *Lonsdalea quercina* subsp. *quercina*, has been identified as the causal agent of 'drippy nut disease' in oak in the USA (Brady et al., 2012). No records can be found in the scientific literature to suggest that either bacterium is present in Canada.

SOURCES: Brady C.L., Cleenwerck I., Denman S., Venter S.N., Rodríguez-Palenzuela P., Coutinho T.A. and De Vos P. (2012) Proposal to reclassify *Brenneria quercina* (Hildebrand & Schroth 1967) Hauben *et al*. 1999 into a novel genus, *Lonsdalea* gen. nov., as *Lonsdalea quercina* comb. nov., descriptions of *Lonsdalea quercina* subsp. *quercina* comb. nov., *Lonsdalea quercina* subsp. *iberica* subsp. nov., and *Lonsdalea quercina* subsp. *britannica* subsp. nov., emendation of the description of the genus *Brenneria*, reclassification of *Dickeya dieffenbachiae* as *Dickeya dadantii* subsp. *dieffenbachiae* comb. nov., and emendation of the description of *Dickeya dadantii*. International Journal of Systematic and Evolutionary Microbiology 62: 1592–1602.

Li, Y., He, W., Ren, F., Guo, L., Chang, J., Cleenwerck, I. Ma, Y. and Wang, H. (2014) A Canker Disease of *Populus x euramericana* in China caused by *Lonsdalea quercina* subsp. *populi*. Plant Disease 98(3): 368-378 DOI 10.1094/PDIS-01-13-0115-RE.

Tóth, T., Lakatos, T. and Koltay, A. (2013) *Lonsdalea quercina* subsp. *populi* subsp. nov., isolated from bark canker of poplar trees. International Journal of Systematic and Evolutionary Microbiology 63: 2309-2313 DOI 10.1099/ijs.0.042911-0.

2 Update: '*Candidatus* Phytoplasma prunorum' (European stone fruit yellow) in apricot orchards in the Czech Republic

A recent study provides an update on the status of '*Candidatus* Phytoplasma prunorum' (European stone fruit yellow) in the Czech Republic where the disease was reported more than 15 years ago. Long-term monitoring of '*Candidatus* Phytoplasma prunorum' in orchards concludes that the disease is an increasing concern for growers. A 50% infection level and an average of 30% of tree die-off (up to 40% in young trees) are reported in apricot orchards even when certified trees are being planted. Although disease symptoms are quite variable, chlorotic leaf-roll was the most common symptom observed in apricots during this study.

'*Candidatus* Phytoplasma prunorum' is known to be present in most European countries and causes important losses in apricot, peach and Japanese plum. It is considered a quarantine pest to Canada.

SOURCE: Nečas, T., Ondrásek, I. and Krska, B. (2015) 'Candidatus Phytoplasma prunorum' (European stone fruit yellow) - a pathogen spreading uncontrollably in apricot orchards in the Czech Republic. Acta Hortic 1105: 131-136 DOI: 10.17660/ActaHortic.2015.1105.19.

Entomology

3 Update: Ash tree resistance to the emerald ash borer

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), is a regulated quarantine pest for Canada, present so far only in parts of Ontario and eastern Quebec. Prohibitions on the movement of ash material and firewood have been implemented to slow the spread of the pest and 'buy time' so that studies researching host resistance of ash to EAB might evolve to a point where treatments are available that could further hinder the pests' movement or save high-value trees.

Villari et al. (2016) recently reviewed the current literature to analyze mechanisms underlying inter- and intraspecific variation in ash resistance to EAB. The review made the following conclusions:

- Manchurian ash is less preferred for adult feeding and oviposition than susceptible North American species and more resistant to larval feeding (Chakraborty et al., 2014; Rigsby et al., 2014).
- Drought stress decreased the resistance of Manchurian ash, but had no effect on constitutive bark phenolics, suggesting that they do not contribute to increased susceptibility in response to drought stress (Chakraborty et al., 2014).
- Application of methyl jasmonate was associated with increased bark concentrations of verbascoside, lignin and/or trypsin inhibitors which decreased larval survival and/or growth in bioassays, suggesting that green, white and black ash possess potential for resistance that is not expressed under natural conditions (Whitehill et al., 2014).

The authors also point to an intriguing find that a very small proportion of green ash have survived in heavily EAB-attacked stands and suggest that these 'lingering ash' could provide material to study resistance traits. A recent study by Koch et al. (2015) investigated intraspecific variation in the 'lingering ash' referred to in an effort to identify specific traits or phenotypes that are likely to be associated with increased ability to survive EAB infestation. Three selections were significantly less preferred for adult feeding, but no specific leaf volatile was associated with reduced preference, and two selections had significant differences in larval development. The results indicate that more than one mechanism is likely responsible for providing resistance in certain ash trees. Koch et al. (2015) suggest continued monitoring and preservation of ash trees that fit the criteria of the 'lingering ash' which could lead to the identification of additional EAB-resistant selections of North American ash species and sources of resistance genes for breeding programs.

SOURCES: Chakraborty, S., Whitehill, J.G.A., Hill, A.L., Opiyo, S.O., Cipollini, D., Herms, D.A. and Bonello, P. (2014) Effects of water availability on emerald ash borer larval performance and phloem phenolics of Manchurian and black ash. Plant, Cell and Environment 37: 1009-1021.

Koch, J.L., Carey, D.W., Mason, M.E., Poland, T.M., and Knight, K.S. (2015) Intraspecific variation in *Fraxinus pennsylvanica* responses to emerald ash borer (*Agrilus planipennis*). New Forests 46: 995-1011.

Villari, C., Herms, D. A., Whitehill, J. G., Cipollini, D. and Bonello, P. (2016) Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer, a model for wood-boring insects that kill angiosperms. New Phytologist 209: 63-79.

Whitehill, J.G.A., Rigsby, C.M. Cipollini, D., Herms, D.A., Bonello, P. (2014) Decreased emergence of emerald ash borer from ash treated with methyl jasmonate is associated with induction of general defense traits and the toxic phenolic compound verbascoside. Oecologia 176: 1047-1059.

4 New Pest: *Agrilus ribesi* goes undetected in North America for a century

The Eurasian species *Agrilus ribesi* was recently reported for the first time from North America (Jendek et al., 2015) and proposed to have caused damage to currants (*Ribes* spp.) in Ontario, previously ascribed to *A. cuprescens* (Garlick, 1940). The discovery was triggered by Garlick's record of black currant, red currant and gooseberry as host plants of *A. cuprescens* in Ontario which were refuted as such in the more recent list of verified host plants (Jendek, 2003; Jendek and Poláková, 2014). The biology of *A. cuprescens,* a notorious pest of *Rubus* and *Rosa,* is well documented, while its development in *Ribes* had never been confirmed, signalling the need for record re-evaluation. All specimens of *A. cuprescens* in the Canadian National Collection were critically examined and 16 of them were re-identified as those of *A. ribesi*.

Morphological diagnostic characters for the two *Agrilus* species are provided in the recent report (Jendek et al., 2015) and complemented with DNA barcodes for four alien *Agrilus* species established in North America (i.e., *A. ribesi, A. cuprescens, A. planipennis* and *A. sulcicollis*) to enable DNA-based identification. Low genetic variability of the North American populations of *A. cuprescens* and *A. ribesi* could indicate a single introduction to North America for each of these species.

SOURCES: Garlick, W.G. (1940) Notes on the rose stem girdler, *Agrilus communis rubicola* Abeille. Canadian Entomologist 72: 21-23.

Jendek, E. (2003) Revision of *Agrilus cuprescens* (Ménétriés, 1832) and related species (Coleoptera: Buprestidae). Zootaxa 317: 1-22.

Jendek, E., Grebennikov, V. and Bocak, L. (2015) Undetected for a century: Palaearctic *Agrilus* Schaefer (Coleoptera: Buprestidae) on currant in North America, with adult morphology, larval biology and DNA barcode. Zootaxa 4034(1): 112-126.

Jendek, E. and Poláková, J. (2014) Host plants of world *Agrilus* (Coleoptera: Buprestidae). A critical review. Springer, Berlin, 706 pp.

5 *Bactra bactrana*, a sedge-feeding leafroller, attacking greenhouse sweet peppers

A recent bulletin reports *Bactra bactana* (Lepidotpera: Tortricidae) attacking sweet peppers, *Capsicum annuum*, for the first time (Roditakis et al., 2015). The infestation was detected in two greenhouses in Southern Crete, Greece, where moth larvae caused typical symptoms of a fruit borer, including small holes on the surface of the peppers and internal damage due to feeding activity. Based on the observed infestation levels of 30% and 15% of fruit in the two greenhouses, *B. bactana* could be considered a potential pest of sweet pepper. Unknown factors are expected to have facilitated the major host shift as the moth coexists with peppers in other parts of Europe without causing damage.

Species from this genus have been used for the control of weeds. This find highlights the need for extensive host plant testing when considering the release of biocontrol agents. Although some associations cannot be predicted, host plants of clear economic value should be considered for inclusion in these tests, even if the range of known hosts of a control agent is narrow.

SOURCE: Roditakis, E., Morin, S. and Baixeras, J. (2015) Is *Bactra bactrana* (Kennel, 1901) a novel pest of sweet peppers? Bulletin of Entomological Research 1-7 DOI:10.1017/S0007485315000917.

Botany

6 First Report: *Orobanche* species parasitizing commercial sunflowers in the U.S.

In September 2014, *Orobanche ludoviciana* (Louisiana broomrape) was found parasitizing the roots of sunflower plants in a commercial sunflower production field in Kimball County, Nebraska. It was the first report of any *Orobanche* species parasitizing commercial sunflowers in the western hemisphere.

Orobanche species are obligate parasitic plants that establish vascular connections to roots of host plants from which they draw nutrients and water. *Orobanche cumana* is a well-known widespread and economically damaging pest of sunflowers in Europe. The species in question, *O. ludoviciana,* is native to North America and is widely dispersed in the Great Plains region. In Canada, it is found in southern British Columbia and the Prairie Provinces and is known to parasitize other members of the Asteraceae family (esp. *Ambrosia* and *Artemisia*) (Scoggan, 1979). The plants have pink stems and purple flowers, and arise from the base of the host plant.

In the affected field in Nebraska, about 30% of sunflowers in 25% of the total area of the field were parasitized by *O. ludoviciana.* The parasitized plants were significantly stunted, with smaller heads and thinner stalks. It is uncertain if yields were impacted. This new finding has raised some concern for sunflower growers in the Great Plains region.

In Canada, sunflowers represent a small but important part of the Prairie agricultural industry. Approximately 35,000 ha of sunflowers are seeded each year, resulting in an annual production of about 67,000 tonnes of sunflower seeds (Statistics Canada, 2015). *Orobanche* species are regulated at the genus level under Canada's *Plant Protection* Act; however, *O. ludoviciana* is an exception because it is a native plant in Canada. Awareness of this plant and its potential to parasitize sunflowers may be important for early detection and management of infections should they occur.

SOURCES: Harveson, R. M., Nelson, A., Mathew, F. M. and Seiler, G. J. (2015) First report of *Orobanche ludoviciana* parasitizing sunflowers. Plant Health Progress doi:10.1094/PHP-BR-15-0043.

Scoggan, H. J. (1979) Flora of Canada. National Museums of Canada, Ottawa.

Statistics Canada. (2015) Tables by subject: Crops and horticulture. Field and special crops. [Online] Available: http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/ind01/l3_920_2024-eng.htm?hili_prim11 [5 Jan 2016].

7 Pathway: Proposed changes to ballast water management – implications for invasive plants and plant pests

Ballast water has long been recognized as a significant vector for the transport and introduction of new invasive species both in Canada and around the world. Ballast is taken on board ships to control their stability and trim, but because it is typically taken up and released in different locations it can facilitate the rapid movement of species, sometimes over large distances. Ballast water is attributed with introducing a number of harmful invasive species in Canada, including zebra mussels, quagga mussels, round goby and spiny water flea in the Great Lakes Region, and others such as the green crab and common periwinkle in coastal areas. Ballast water and associated sediment can also be a vector for aquatic and wetland plants, and associated pests and diseases.

In Canada, ballast water management (BWM) guidelines have been in place since the 1980s, and statutory regulations under the *Canada Shipping Act* have been in effect since 2006. Current regulations apply to all Canadian vessels worldwide, as well as non-Canadian vessels operating in waters under Canadian jurisdiction. They require ballast water to be treated, exchanged, transferred to a reception facility or retained on board, to minimize the possibility of spreading harmful organisms. By far the most common management method used in Canada and around the world is mid-ocean exchange (MOE), which works on the principle of differences in water conditions (primarily salinity) between source and exchange locations. For example, many ships take up and release ballast in freshwater ports, crossing the ocean in between. Exchanging the ballast mid-ocean exposes freshwater organisms to intolerable salinity levels, and ensures that ballast exchange occurs between ecologically different zones, thus lowering the risk of invasion. However, there are a number of limitations to existing regulations and the practice of MOE, particularly for vessels moving and practicing MOE within or between marine systems where salinity levels are similar.

In 2010, Canada ratified the International Maritime Organization (IMO)'s *International Convention for the Control and Management of Ships' Ballast Water and Sediments* (BWMC). This was the first attempt at an international, legally binding legislation for BWM, and will come into effect twelve months after ratification by 30 member states representing at least 35% of the world shipping tonnage (currently at 43 states and 32.54%). The convention introduces new requirements for on-board testing and concentration-based discharge standards (the "D-2 standards"), although the effectiveness of a number of treatment systems certified under this program have been called into question. Currently, Transport Canada is deliberating how to proceed with implementation of the IMO D-2 standards, and the exact nature and timing of any new BWM requirements at the national level remains unclear. In the meantime, Transport Canada has initiated discussions with the CFIA about possible implications of these new requirements for the risk associated with plants and plant pests that could be transported in ballast water or associated sediment.

SOURCES: Cohen, A. N. and Dobbs, F. C. (2015) Failure of the public health testing program for ballast water treatment systems. Marine Pollution Bulletin 91(1): 29-34.

Mills, E. L., Leach, J. H., Carlton, J. T. and Secor, C. L. (1993) Exotic species in the Great Lakes: A history of biotic crises and anthropogenic introductions. Journal of Great Lakes Research 19(1): 1-54.

Scriven, D. R., DiBacco, C., Locke, A. and Therriault, T. W. (2015) Ballast water management in Canada: A historical perspective and implications for the future. Marine Policy 59: 121-133.

Biotechnology

8 Review: The global outlook for genetically modified crops

The development and cultivation of genetically modified (GM) crops is increasing on a global scale. The global pipeline of GM crops is evolving, and this has implications for the international trade of agricultural commodities. In 2008, the European Commission's Joint Research Centre (JRC) examined the global situation of GM crops in development (Stein and Rodríguez-Cerezo, 2009). A follow-up document published in January 2016

(Parisi et al., 2016) reported that GM events nearly doubled from 2008 to 2015.

Diversity of GM crop types and traits are increasing at all stages of development. Crop types are currently dominated by maize, cotton, soybean and oilseed rape. However, biomass for liquid fuels and industrial products is becoming an important sub-sector of GM crops, driven by market demand. Rice and potatoes are also major upcoming GM crops, and cereals, fruits and vegetables are also under development in Brazil, India and China. Public developers in India and China are becoming increasingly active in GM crop development. New, smaller companies are emerging in the United States, India and other parts of Asia. Although herbicide-tolerance and insect-tolerance are the most dominant traits for GM crops, herbicide-tolerance traits are shifting from glyphosate and glufosinate to other active ingredients such as sulfonylurea, 2,4-D, dicamba, isoxaflutole and oxynil. New and emerging traits are being developed worldwide, particularly in Asia, including insect-resistant eggplant (India), insect-resistant poplar (China) and virus-resistant bean (Indonesia). Important traits in African countries include insect and disease tolerance, abiotic stress tolerance (i.e., drought) and biofortification for human nutrition in crops such as banana, cowpea and rice. The development of crops with more than one improved agronomic trait is becoming increasingly common. Known as 'stacked varieties', these may be developed using molecular tools or through conventional breeding of two or more plant lines with GM events. Stacked varieties are projected to play a major role in the development of upcoming GM crops. Unfortunately, there are large discrepancies in the regulatory treatment of stacked varieties across countries, which can result in asynchronous authorization.

In summary, the current trend towards increasing development and cultivation of GM crops in diverse geographic regions is projected to continue. Thus, there is a strong need for international dialogue to minimize the negative effects of asynchronous authorization on global agricultural trade.

SOURCES: Parisi, C., Tillie, P. and Rodríguez-Cerezo, E. (2016) The global pipeline of GM crops out to 2020. Nature Biotechnology 34(1): 31-36.

Stein, A.J. and Rodríguez-Cerezo, E. (2009) The global pipeline of new GM crops. Implications of asynchronous approval for international trade. European Commission, Joint Research Centre.

9 New Technology: The patent battle over CRISPR-Cas9 techniques

We often remark about the great potential of emerging technologies, but rarely do we observe them moving quickly from discovery to commercialization. CRISPR gene editing systems are certainly bucking this trend. Since 2012, when CRISPRs were first engineered to target specific genetic sequences *in vitro*, the technology has been used to successfully edit bacterial, fungal, animal and plant genomic sequences *in vivo*. Moreover, an intense foundational technology patent battle has emerged between scientists at the University of California, Berkley and the Broad Institute of MIT and Harvard. The outcome of this patent battle will influence hundreds of millions of dollars already committed to CRISPR-based companies.

The patent dispute traces back to March 15, 2013 when Jennifer Doudna and Emmanuelle Charpentier, of UC Berkley and the Max Plank Institute, respectively, filed for a joint CRISPR-Cas9 technique patent with the United States Patent and Trademark Office (USPTO). In October 2013, Feng Zhang of the Broad Institute of MIT filed to protect his CRISPR-Cas9 technique using an expedited review. The Zhang patent was granted in April 2014, while the Doudna-Charpentier application was still being processed 2 years later. In January, 2016 the USPTO decided to review who should have been awarded the CRISPR-Cas9 patent; Doudna-Charpentier or Zhang. This process, called patent interference, functions much like a court case and will likely see both Doudna and Zhang deposed under oath with evidence used to establish what group invented the technique first. Many expect that laboratory notes will play a large part in establishing the timeline.

The outcome of these proceedings will be important for the agricultural biotechnology sector. Doudna's biotechnology start-up, Caribou Biosciences, recently announced a CRISPR-Cas9 patent sharing agreement with DuPont-Pioneer. Both groups possess CRISPR-Cas9 patents, and this partnership allows access to each other's CRISPR intellectual property. This partnership also divvies up the agricultural crop space; DuPont will develop crops like maize, soybean and canola, while Caribou Biosciences will be responsible for fruits and vegetables. If Doudna and Charpentier are unsuccessful in their interference challenge, then it's reasonable to expect that this Caribou Bioscience – DuPont partnership will have to restructure significantly, if it continues to exist at all.

For Canadian regulators, the outcome of this patent dispute may be interesting, but is unlikely to affect daily activities. Moreover, despite there being significant uncertainty over how CRISPR technologies will be treated by worldwide regulatory agencies, Canada's product based regulatory system is well positioned to address incoming Plants with Novel Traits derived from CRISPR-Cas9 tool sets.

SOURCES: Ledford, H. (2016) Bitter fight over CRISPR patent heats up. Nature 529, 265 doi:10.1038/nature.2015.17961.

Grushkin, D. (2016) DuPont in CRISPR-Cas patent land grab, Nature Biotechnology 34:1, 13 doi:10.1038/nbt0116-13.

Acknowledgments

Thanks to the following CFIA staff who contributed to this edition of the Plant Science Scan: K. Castro, J. Dalton, M. Damus, B. Day, J.-F. Dubuc, V. Grebennikov, D. Holden, W. Laviolette, D. Levac, A. Sissons, C. Wilson and L. Vyvey.

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