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

PLANT SCIENCE SCAN

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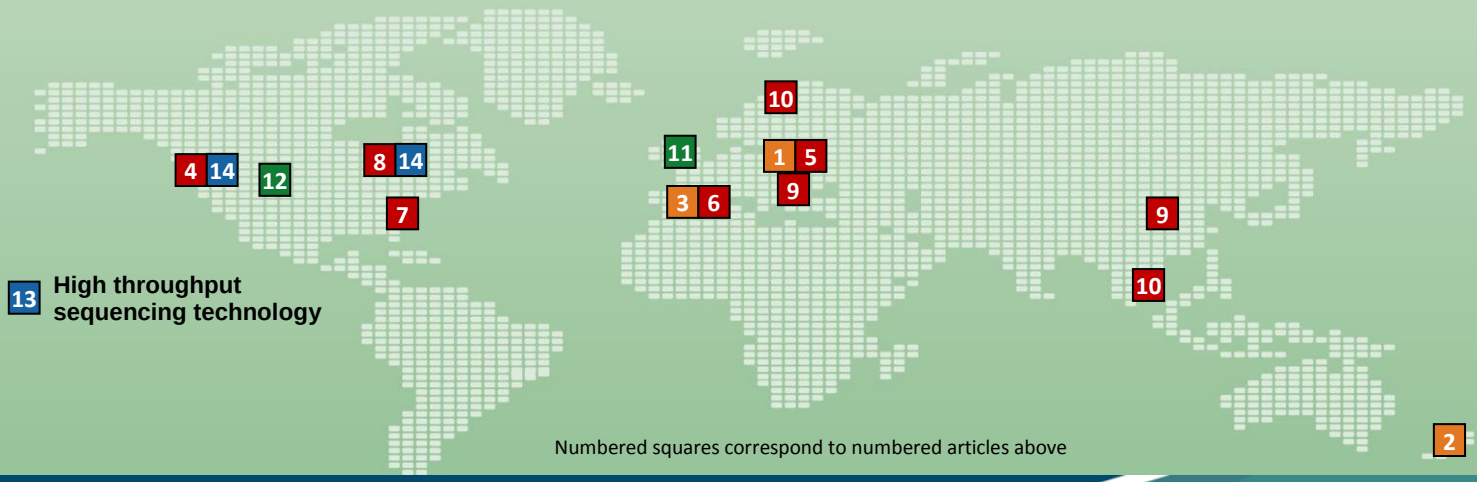
BACKGROUND: The Plant Health Science Directorate of the Canadian Food Inspection Agency routinely scans external sources to identify information that might be of possible regulatory significance or interest to Canada's national plant health. This Plant Science Scan report was prepared by the Canadian 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



Pathology

1 New hosts of the phytoplasma '*Candidatus* Phytoplasma mali', causal agent of apple proliferation disease

In a 2017 survey in Lithuania, phytoplasma-like symptoms were observed in plants of *Forsythia suspensa* (leaf reddening), *Spiraea vanhouttei* (decline) and *Viburnum lantana* (witches'-broom) in a private garden in the district of Kaunas. Molecular analyses confirmed that the causal agent was '*Candidatus* Phytoplasma mali'. This is the first report of these three plant species infected by this phytoplasma.

'*Candidatus* Phytoplasma mali' belongs to the 16SrX-A subgroup and causes witches' broom, phyllody, reduced fruit quality, and severe yield loss in *Malus* spp. (apple). It is also associated with other economically important plants such as *Prunus* spp. (stone fruit), *Vitis vinifera* (grape), *Corylus avellana* (European hazelnut), and *Lilium* spp. (lily).

'*Candidatus* Phytoplasma mali' has been reported from Europe, Tunisia and the Middle East. In Canada, a suspected detection of '*Candidatus* Phytoplasma mali' was reported in apple trees in an orchard in Nova Scotia in 2013 but extensive follow-up surveys and further analyses of DNA sequences confirmed this was an invalid detection. Therefore, this phytoplasma has never occurred in Canada.

'*Candidatus* Phytoplasma mali', which is insect-vectored, is a regulated pest for Canada; the new host records represent new pathways for the introduction of this phytoplasma.

SOURCES: Abraitienë, A., Bevilacqua, A., Scarafoni, A. and QUAGLINO, F. 2018. First report of *Forsythia suspensa*, *Spiraea vanhouttei* and *Viburnum lantana* as new natural plant hosts of '*Candidatus* Phytoplasma mali', the causal agent of apple proliferation disease, in Lithuania. Plant Disease, published online https://apsjournals.apsnet.org/doi/10.1094/PDIS-01-18-0090-PDN.

PestLens. 2013. First report of the phytoplasma '*Candidatus* Phytoplasma mali', causal agent of apple proliferation disease, in Canada.

CFIA. 2013. Update for Stakeholders: Phytoplasma in an apple orchard in Nova Scotia. http://www.inspection.gc.ca/plants/plant-pests-invasive-species/diseases/apple-proliferation-phytoplasma/update-for-stakeholders/eng/1382553102841/1382553104248

2 Preventing the introduction of invasive pathogens through plant health biosecurity measures

In a recent study, the rates of establishment of non-native pathogen species in New Zealand were analyzed from 1881 to 2012 and compared to import trade volumes and international travellers. The analysis revealed that pathogen arrival rates had constantly increased from 1880 to about 1980 but stabilized thereafter. The data suggest a correlation between pathogen arrival rates and import trade volumes during that period; however, no correlation was found with international travellers.

A more detailed analysis was performed for the four primary production sectors. For the pasture and crop sectors, pathogen arrival rates increased until the 1960s, followed by a decrease which coincides with improved biosecurity measures implemented after the 1950s. In contrast, an ongoing increase in pathogen arrival rates was noted for the forestry and fruit trees sectors. Less effective biosecurity measures, delayed peak survey efforts (1960s for pasture and crop species; 1980s for fruit trees; 2000 for forestry species),

and an increased focus on response to pest incursions rather than on the prevention of new pest introductions may have contributed to the steady increase in pathogen arrival rates in these sectors. The authors claim that this finding is the first evidence that investment in biosecurity would be effective to limit pathogen entry and establishment in a context of increasing trade.

SOURCE: Sikes, B. A., Bufford, J. L., Hulme, P. E., Cooper, J. A., Johnston, P. R. and Duncan, R. P. 2018. Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. PLOS Biology 16(5):e2006025.

3 *Xanthomonas prunicola*, a new species causing bacterial canker of nectarine in Spain

A new species of *Xanthomonas*, *X. prunicola* sp. nov., was isolated from *Prunus persica* var. *nectarina* (nectarine). Strains were collected from symptomatic trees found in Murcia, Spain. The colonies were originally thought to be that of *Xanthomonas arbicola* pv. *pruni* which causes bacterial spot disease of stone fruit. Phenotypic and molecular characterization determined that these isolates were members of the genus *Xanthomonas;* however characteristics did not match any known species. The results of molecular analysis confirmed that they formed a homogenously distinct group and the name *Xanthomonas prunicola* has been proposed. Pathogenicity was confirmed by infecting *P. persica* leaves with bacterial suspensions.

There is very little information on the biology (e.g. host range) and impact of *Xanthomonas prunicola*. Therefore, more information is needed to evaluate its potential economic impact to the stone fruit industry should it be introduced into Canada. Of note, Canada produces 21,124 metric tonnes of peaches on over 2567 hectares with a farm gate value of $35 million dollars (AAFC 2016).

SOURCES: López, M. M. et al. 2018. *Xanthomonas prunicola* sp. nov., a novel pathogen that affects nectarine (*Prunus persica* var. *nectarina*) trees. International Journal of Systematic and Evolutionary Microbiology. 68:1857-1866.

Agriculture and Agri-Food Canada (AAFC). 2016. Statistical Overview of the Canadian Fruit Industry – 2016. On-line at: http://www.agr.gc.ca/eng/industry-markets-and-trade/market-information-by-sector/horticulture/horticulture-sector-reports/statistical-overview-of-the-canadian-fruit-industry-2016/?id=1499785006783

Entomology

4 Non-native shield bug arrives in Canada

This publication records the presence of the juniper shield bug species, *Cyphostethus tristriatus*, in North America for the first time. The primary author was contacted regarding potential signs of harm to host plants, and none were noted. It was the most common shield bug on the host plants, however, and seemed to displace native species. It is not recorded as a pest in Europe, although its numbers are increasing, as well as its range. This insect does not yet seem to be an 'up-and-coming pest', but it bears watching, because so far it appears to be in limited distribution in Canada. Its presence near a biodiversity museum on a university campus suggests, but does not prove, a potential means of entry.

SOURCE: Ratzlaff, C. G. and Scudder, G. G. E. 2018. First records of the juniper shield bug, *Cyphostethus tristriatus* (Fabricius, 1787) (Hemiptera: Acanthosomatidae), in North America. Pan-Pacific Entomologist 94(2):67-74. Pan-Pacific Entomologist 94(2):67-74.

5 Development of a modelling tool to assess the risk of pest transfer from European plant imports to domestic production

Inspection of plant imports is one of the safeguards used by National Plant Protection Organizations for reducing pest introductions but capacity constraints limit inspection efforts. This is why many of these organizations, including the Canadian Food Inspection Agency, have started looking at efficiencies in inspection efforts like the one described in this paper.

SOURCES: EC. (2004). Commission Regulation EC/1756/2004 Specifying the detailed conditions for the evidence required and the criteria for the type and level of the reduction of the plant health checks of certain plants, plant products or other objects listed in Part B of Annex V to Council Directive 2000/29/EC. Source, http://ec.europa.eu/food/plant/plant_health_biosecurity/non_eu_trade/less_frequent_checks_en [Accessed, July 30, 2018].

Holt, J., van der Gaag, D. J., Leach, A. W., Loomans, A. J. M., and Mumford, J. D. 2017. Model of the probability of pest transfer from imported fruit, cut flowers or vegetable produce. Bulletin OEPP/EPPO Bulletin, 47 (2): 227–230.

6 First record of the string cottony scale *Takahashia japonica* in Europe and its establishment in Northern Italy

Information on *Takahashia japonica* is scarce. This species was described from Japan (Tokyo) on *Morus.* sp. It was historically found also upon citrus there, but as an occasional pest with little or no consequence from the economic point of view (Clausen, 1927). It is presently recorded also in China (Hunan, Shanxi) and South Korea. This article from Italy is reporting an invasive behaviour. The pest has spread in an area of about 42 square kilometres in a few years. In several cases, the infestation was very high with branches and twigs covered by eggsacs. It has also attacked four new plant hosts (*Acer pseudoplatanus, Albizia julibrissin*, *Carpinus betulus and Celtis australis*). Some of the natural hosts include *Morus nigra*, *Morus alba*, *Cydonia oblonga, Prunus cerasifera*, and *Prunus salicina*, which are of economic importance. Scale insects are among the most common invaders of new geographic areas in Europe.

The pest is also listed in a PHRA 97-49 (Insects potentially associated with ash species (*Fraxinus* spp.) from off continental sources), but the information was insufficient to make a conclusion about its importance. Considering the current pest distribution, pest establishment could be possible at least in British Columbia.

SOURCES: Limonta, L. Pellizzari, G., 2018. First record of the string cottony scale *Takahashia japonica* in Europe and its establishment in Northern Italy. Bulletin of Insectology 71 (1): 159-160.

Clausen, C., 1927. The citrus insects of Japan. USDA Technical bulletin No.15 1-16pp.

7 First records of *Cyclorhipidion fukiense* (Eggers) (Coleoptera: Curculionidae: Scolytinae: Xyleborini), an ambrosia beetle native to Asia, in North America

Ambrosia beetles and bark beetles form a highly diverse group of small wood-boring beetles, belonging to the subfamilies *Scolytinae* and *Platypodinae* (Coleoptera: Curculionidae). Ambrosia beetles mainly colonize trees that are either dead or decaying; however a few species are able to attack live and healthy trees and could become invasive and economically important.

There are over 60 species of non-native ambrosia and bark beetles that have been recorded in North America. Of the genus

Cyclorhipidion, two non-native species, *bodoanum* and *pelliculosum*, are known to occur in North America. A third species, *fukiense,* native to Asia, has recently been found in North America and this paper is the first report of its presence in this region. The main hosts of this beetle belong to the family *Fagaceae* and other hosts include species of *Castanea*, *Quercus and Castanopsis*. Association with hosts in North America is currently unknown as this species has only been found in traps. The information presented in this paper is relevant to the CFIA because many of the hosts of *C. fukiense* are important plants in Canada and if this species was to enter Canada, it could have the potential to become invasive and cause economic harm.

SOURCE: Hoebeke, E. R., Rabaglia, R. J., Knizek, M., & Weaver, J. S. (2018). First records of *Cyclorhipidion fukiense* (Eggers) (Coleoptera: Curculionidae: Scolytinae: Xyleborini), an ambrosia beetle native to Asia, in North America. Zootaxa, 4394(2), 243-250.

8 Five year observations of the impact of *Sirex noctilio* in Ontario Pine Forests

Sirex noctilio is present in Canada and is a regulated pest. It is native to Europe and Asia and was introduced into North America around 2005. It is a major pest of pine trees. A decade after its discovery, this pest has not been an aggressive killer of pine tree forests in its invaded areas in North America. But concern is still warranted that this invasive pest has potential to become more aggressive as it spreads southwards, or as environmental conditions change, because it has caused extensive pine losses on other continents that it has invaded.

SOURCE: Haavik, L. J., Dodds, K. J. and Allison, J. D., 2018. *Sirex noctilio* (Hymenoptera: Siricidae) in Ontario (Canada) pine forests: observations over five years. The Canadian Entomologist, 150(3), 347-360.

9 Sentinel plants find the unknown unknowns in horticulture imports

This study highlights a few things of importance to Canada: 1) risk assessments are important before allowing the importation of new plants, especially for the horticultural trade, and 2) the number of 'unknown unknowns' is very great, even with the volume of literature available nowadays, 3) the best way to solve this problem is through partnerships with the importing industry, the exporting countries, and possibly with international partners with the same interests. Setting up a series of sentinel stations would be an excellent collaboration with international partners. The vast majority of Canada's live plant imports come from the United States, with others coming from Central America, Mexico, France, the Netherlands and parts of the Middle East and Africa. While the overall risk to Canada from plant importations from the U.S. is probably very low, importations from other countries, especially those with a similar climate to Canada, could bring pests science is not even aware of.

SOURCE: Kenis, M., Li, H., Fan, J. T., Courtial, B., Auger-Rozenberg, M. A., Yart, A., Eschen, R. and Roques, A. 2018. Sentinel nurseries to assess the phytosanitary risks from insect pests on importations of live plants. Scientific Reports 8(1):11217 | DOI:10.1038/s41598-018-29551-y.

CFIA Scientist Publication

10 Advancements in the complexity of the genus *Agrilus*

The jewel beetle genus *Agrilus* is the largest known genus with over 3,000 species and potentially thousands of unnamed species. All species are known to be phytophygous and some are known to have significant biological and economic impacts when introduced to new areas, especially those that are free of natural enemies. For example, introduction of the emerald ash borer (*A. planipennis*) to North America is estimated to have had an economic impact of greater than 10 billion USD.

Due to the increasing number of non-native species, there is a need to develop ways for fast and reliable identification such as DNA-based pest diagnostic tools. A recent study describes the development of the first DNA reference database for over 100 *Agrilus* species from the Northern Hemisphere, based on the mitochondrial markers *cox1–5'* (DNA barcode fragment), *cox1–3'*, and *rrnL*. The records are publicly available in the Barcode of Life Data System. This study also describes a first attempt to assemble the phylogeny of *Agrilus*, making it the first dataset capable of identifying an unknown specimen from the northern temperate zone.

A second study describes 20 new *Agrilus* species from the Oriental Region.

SOURCES: Kelnarova, I., Jendek, E., Grebennikov V.V., Bocak, L. 2018. First molecular phylogeny of *Agrilus* (Coleoptera: Buprestidae), the largest genus on Earth, with DNA barcode database for forestry pest diagnostics. Bulletin of Entomological Research, May 22:1-12.

Jendek, E., Grebennikov V.V. 2018. Twenty new species of *Agrilus* (Coleoptera: Buprestidae: Agrilinae) from the Oriental Region. Zootaxa, 4429(1): 107–131.

Botany

11 A citizen science approach to prioritizing invasive ornamental plants

Citizen science presents a tremendous opportunity to complement regulatory approaches for early detection of invasive alien pests. However, with respect to plants for planting, the public is presented with a quandary as many of the desirable characteristics of ornamental plants (e.g., ease of establishment and propagation, vigorous growth, long flowering habit, wide tolerance to varying edaphic conditions, stress hardiness, etc.) are the same characteristics that enable some species to establish, spread and have negative economic or environmental impacts.

Recognizing that gardeners could be a valuable resource for determining new and potentially problematic ornamental species, a recent British study used an online-survey to ask gardeners to report on ornamental plants that were spreading and/or difficult to control in their gardens. The introduction to the survey first acknowledged the important contribution and value of non-native ornamental plants to gardeners' enjoyment of their gardens. It then shifted to a short explanation of how ornamentals may sometimes become problematic when they escape from gardens and become invasive, for example by causing negative impacts to biodiversity. Strategically, the survey design then encouraged public engagement by informing gardeners how they could play a crucial role in identifying ornamental plants that are potentially invasive.

Fifty-six British gardeners from across the UK responded with 201 records of plants that were spreading in their gardens or difficult to control; these included 121 species, of which 17 were native and 104 were non-native. Interestingly, about a third of the species reported were not previously known to be widespread outside of cultivation and eight species were not known to be present at all outside of cultivation in Britain. The authors argue that engaging the public in this way could help to identify potentially invasive species from among the myriad of non-native ornamental plant species on the market, and this information could be used to prioritize risk assessments and focus early detection efforts as well as preventative measures.

SOURCE: Dehnen-Schmutz, K. and Controy, J. 2018. Working with gardeners to identify potentially invasive ornamental garden plants: testing a citizen science approach. Biological Invasions.

12 The invasive potential of Eurasian haskap (*Lonicera caerulea* L.)

Lonicera caerulea L. is a complex of deciduous, fruit-producing shrubs of circumboreal regions of the world. Many diverse cultivars have been introduced in North America, marketed as haskap, honeyberry, or sweetberry honeysuckle. Breeding efforts have been concentrated in Oregon and Saskatchewan. *L. caerulea* is taxonomically complex and includes a number of subspecies and varieties. Two subspecies, subsp. *villosa* and subsp. *cauriana,* are native to North America; in Canada, subsp. *villosa* is widespread from Alberta to the Atlantic, and northward to Nunavut (reported as *L. villosa*, Brouillet et al. 2010+). However, cultivars that are currently commercially available in North America are grown from Eurasian germplasm. Improved hybrid cultivars are also under development, which involve crosses between plants of multiple provenances, including those of Canadian origin.

Many publications promote Eurasian haskap for home landscapes and either fail to discuss the species' invasive potential or deem it non-invasive. The plants can produce 50,000 – 100,000 seeds annually and multiply rapidly. New hybrid cultivars may promote faster growth, higher yield, greater disease resistance, and better adaptability. The species is considered highly invasive in the forested habitats of Norway and is greatly affecting their native flora. The plant has naturalized in at least one North American location already and has the potential to further establish in northern forests with shady understories. Based on the results of two weed risk assessment tools, Eurasian *L. caerulea* poses a 'high risk' of becoming a major invader of North America.

There are still several unanswered questions regarding *L. caerulea* and its invasive potential in North America. For instance, taxonomic research is ongoing and it is unclear whether the species may be considered native in North America. As well, it is unknown if Eurasian cultivars will demonstrate better fitness than native relatives (i.e., subsp. *villosa* and subsp. *cauriana*). It is also unknown if native populations may suffer population declines or extinctions due to the spread of introduced alleles from Eurasian cultivars. To gain a better understanding of the potential risks *L. caerulea* presents to North America, more research needs to be conducted on taxonomy, the invasive attributes of hybrids, and the genetic issues associated with cultivar introductions.

SOURCES: Brouillet, L., Coursol, F., Meades, S. J., Favreau, M., Anions, M., Bélisle, P. and Desmet, P. 2010+. VASCAN, the database of vascular plants of Canada. [Online] Available: http://data.canadensys.net/vascan/ [2018].

Peterson, B. J., Stack, L. B. and Hayes, D. J. 2018. What do we know about the invasive potential of *Lonicera caerulea* L. cultivars in North America? Acta Horticulturae 1191: 129-137.

Advances in laboratory diagnostics

CFIA Scientist Publication

13 High-throughput sequencing technologies for plant pest diagnosis: challenges and opportunities

High-throughput sequencing (HTS)—also known commonly as next-generation sequencing or NGS—has revolutionized many fields of biology, so unsurprisingly, it has the potential to do the same for plant pest diagnosis. Although HTS for plant viruses is more advanced (Rott et al. 2017), its general applicability for plant pest diagnosis is becoming more common (Martin et al. 2016).

As in other fields, there are challenges and opportunities, which are the focus of the recent paper by Olmos et al. (2018). After a summary of HTS, the authors highlight its general main advantages, chief amongst these being the increased speed of detection results, a potentially crucial factor in plant pest detection and control. HTS analyses also allow for greater number of tests (ie. sample size), which increases the confidence level of diagnosis. The range of sequence results obtained from HTS means that it is good both for targeted and broad-spectrum screening. With the larger amount of molecular data generated by HTS comes with an increased rate of serendipitous detection of non-target plant pests, depending on the quality and degree of completeness of the sequence database against which the generated sequences are compared. As well, with expansion of databases will come the ability to do post hoc "bioinformatic surveys" on previously collected HTS data. As well, the larger sequence datasets generated allow for a greater understanding of the genetic diversity of a detected plant pest.

The authors specifically highlight 4 areas in which the use of HTS will benefit diagnostics: "1) understanding the status of a pest in a region through surveillance programmes, 2) certifying nuclear stock and plant propagation material, 3) (post-entry) quarantine testing to prevent the introduction of pests into a country or area, and 4) monitoring of imported commodities for new potential risks."

For each of the abovementioned opportunities, there is a corresponding challenge, amongst others. Many of these concern how the new methods fit into an international regulatory framework. Besides the obvious challenges—infrastructure requirements, the learning curve for new methods, greater complexity of bioinformatics analyses—there are some challenges that are unique to plant pest diagnostics. Results of proof-of-concept testing will be required for decisions on adoption of HTS methods by international regulators. Sampling and laboratory guidelines will have to be developed and tested. The increased detection of non-target pests will be one of many new challenges for regulators, especially in cases where multiple pathogens are detected in a single crop/plant situation. A final example of new challenges to regulators brought about by HTS-based surveys will be whether novel findings reflect a new incursion or a previously undetected pest. This will be extremely difficult to determine without

extensive baseline surveys - a significant challenge in the Canadian context given the size of the country compared to the survey resources available.

SOURCES: Rott, M., Xiang, Y., Boyes, I., Belton, M., Saeed, H., Kesanakurti, P., Hayes, S., Lawrence, T., Birch, C., Bhagwat, B., Rast, H. 2017. Application of next generation sequencing for diagnostic testing of tree fruit viruses and viroids. Plant Disease 101: 1489-1499. DOI: 10.1094/PDIS-03-17-0306-RE

Martin, R. R., Constable, F., Tzanetakis, I. E. 2016. Quarantine regulations and the impact of modern detection methods. Annual Review of Phytopathology 54: 189-205. DOI: 10.1146/annurev-phyto-080615-100105

Olmos, A., Boonham, N., Candresse, T., Gentit, P., Giovani, B., Kutnjak, D., … Massart, S. 2018. High-throughput sequencing technologies for plant pest diagnosis: challenges and opportunities. Bulletin OEPP/EPPO Bulletin 48(2): 219-224. DOI: 10.1111/epp.12472

CFIA Scientist Publication

14 New screening method for exotic forest pathogens using metagenomics

Human activities such as agriculture, landscape disposal and international trade pose a significant threat to Canadian natural resources such as forest trees. This study addresses a growing problem for plant health, namely the introduction and spread of fungal pathogens often responsible for disease outbreaks resulting in extensive forest decline. It is known that the earlier the detection of phytopathogenic propagules, the lesser the chance of irreversible damage occurring. Considering that fungi cannot be baited with any kind of attractant (e.g. insects can be trapped using semiochemicals), an alternative approach to assess the presence of potential invasive aliens species spores was evaluated. Given the need, Tremblay et al. (2018) developed a quicker and higher-throughput method for regulated plant pathogens. Collaborators from the Canadian Food Inspection Agency, Natural Resources Canada and Université Laval presented how their 3-year, nationwide sample collection strategy coupled to next-generation sequencing (NGS) (i.e. metagenomics) can help provide guidance to phytopathology stakeholders such as regulatory agencies responsible for surveillance surveys. From a total of 398 samples obtained from three different air samplers, baited insect traps and soil samples, several regulated invasive species were monitored near high-risk areas. A custom bioinformatics pipeline was established to analyse the metagenomics data including indexed samples and three disparate genic regions. Several *Phytophthora* (pseudofungi) species were detected by NGS and confirmed using a standardised qPCR assay. The metagenomics workflow allowed for the detection of the target species *Heterobasidion annosum* s.s (fungi) in Quebec and Ontario. As well, sequences within the *H. annosum* s.l. species complex (*H. abietinum*/*H. parviporum*) (fungi) were also detected in British Columbia. It was demonstrated that this new methodology for screening target species using a variety of sampling techniques and NGS has the potential to increase survey capacity and detection sensitivity, reduce hands-on time and costs, and assist regulatory agencies in identifying ports of entry. Considering that early detection and prevention are the keys in mitigating invasive species damage, this method represents a substantial asset in plant pathology management.

SOURCE: Tremblay, É.D., Duceppe, M.O., Bérubé, J.A., Kimoto, T., Lemieux, C., Bilodeau, G.J. (In press). Screening for exotic forest pathogens to increase survey capacity using metagenomics. Phytopathology DOI: 10.1094/PHYTO-02-18-0028-R

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