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PLANT SCIENCE SCAN

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

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Canada

Pathology

1 First Report: Cherry leaf roll virus from *Malus*

Using molecular testing and phylogenetic analysis, Cherry leaf roll virus (CLRV) was recently identified during a survey of apples trees in New Zealand. Sap from trees testing positive for CLRV was used to inoculate *Nicotiana occidentalis* plants resulting in symptoms of yellow mottling; molecular tests confirmed CLRV from infected plants. The virulence of CLRV from *Malus* is still unknown, and its economic impact on *Malus* species has yet to be determined. According to the authors, this study may represent the first finding of CLRV in apple.

Cherry leaf roll virus is a nepovirus with a wide host range including many horticultural plants. It has been reported to cause plant death in cherry, elderberry, olive, raspberry, rhubarb, walnut and ornamental species. Cherry leaf roll virus is listed by Canada as a regulated pest in *Prunus* propagative materials.

SOURCE:
Woo, E. N. Y., Clover, G. R. G., and M.N. Pearson, 2012. First report of Cherry leaf roll virus (CLRV) in *Malus domestica*. Australasian Plant Disease Notes 7(1): 151-156.

2 Update: Investigating a possible soil phase for *Phytophthora ramorum*

A recent study examined the presence and persistence of *P. ramorum* in root balls of Rhododendron plants using a novel baiting test which was non-destructive, sensitive and applicable to a high sample number. *P. ramorum* was isolated from Rhododendron root balls 8 months after inoculation while above ground plant parts remained symptomless. In addition, *P. ramorum* was identified from root balls of symptomless Rhododendron plants in commercial nurseries. These results suggested that *P. ramorum* survives latently in root balls without causing visible symptoms.

A second part of the study evaluated the survival of infected leaf tissue in a rootless potting medium and forest substrate. Results indicated there was a seasonal effect with lower recovery in the summer and autumn and higher recovery during and after the winter. *P. ramorum* was identified from forest substrate 33 months after burial showing the ability of *P. ramorum* to survive long term in rootless substrates. These results support recent evidence suggesting *P. ramorum* has a soil phase and that its presence in symptomless root balls could potentially contribute to latent spread of this pathogen between nurseries. The non-destructive and simple baiting technique for detecting *P. ramorum* in growing media used in this study could be applicable to a relatively large number of plants, thus offering a potentially valuable regulatory or industry tool for testing plants for the presence of *Phytophthora* species in root balls.

SOURCE:
Vercauteren, A., Riedel, M., Maes, M., Werres, S. and K. Heungens, 2013. Survival of *Phytophthora ramorum* in Rhododendron root balls and in rootless substrates. Plant Pathology 62 (1):166–176.

3 New Host & Distribution Records: *Gaultheria procumbens* and *Myristica fragrans* - newly identified hosts for *Phytophthora ramorum*; First report of *P. ramorum* from India

Phytophthora ramorum was recently identified from *Myristica fragrans* (nutmeg) in several regions in India (Matthew and Beena 2012). This may constitute both a new host and a new distribution record for the pathogen. Infected plants had severe defoliation and shoot rot.

In addition, *Gaultheria procumbens* (eastern teaberry, checkerberry, boxberry, or American wintergreen) was also recently identified as a host for the pathogen in the U.S. (California Oak Mortality Task Force 2013).

The CFIA's list of host plants regulated for *P. ramorum* can be found at http://www.inspection.gc.ca/english/plaveg/protect/dir/sodspe.shtml. *Gaultheria shallon*, Oregon winterberry, is already regulated but *G. procumbens*, a widespread native of northeastern North America, is not. *Myristica fragrans* is not currently on the list of host plants regulated for *P. ramorum.*

SOURCE:
California Oak Mortality Task Force Report, February 2013.

Mathew, S. K. and S. Beena, 2012. A new record of *Phytophthora ramorum* causing leaf fall and shoot rot of nutmeg (*Myristica fragrans*). Journal of Mycology and Plant Pathology 42(4):529-530.

4 Update: Seasonal changes of flavescence dorée phytoplasma in grapevine

Observations from two vineyards in Slovenia showed that flavescence dorée (FD) phytoplasma-infected plants had reduced growth, fewer flower clusters, fewer berry clusters, and berry withering; some of the severely infected plants had no grape clusters. At one site there was a 40 fold increase in FD infected plants detected over the course of the study. The authors proposed this was partially due to the lack of control methods applied for the FD insect vector, *Scaphoideus titanus*. Molecular analysis showed there were increased levels of FD phytoplasma found within leaves and berries over the growing season. The study also demonstrated the successful detection of FD from symptomless tissue early in the growing season.

Flavescence dorée phytoplasma causes an economically significant disease of grapevine in parts of Europe. It is considered a quarantine pest to Canada. Knowledge gained from this study could be of value in helping to develop surveillance and sampling strategies for the pathogen, should it be introduced into Canada.

SOURCE:

Prezelj, N., Nikolić, P., Gruden, K., Ravnikar, M. and M. Dermastia, 2013. Spatiotemporal distribution of flavescence dorée phytoplasma in grapevine. Plant Pathology.

5 First Report: Bleeding canker of horse chestnut in the Czech Republic

First identified in India, bleeding canker of horse chestnut, caused by the bacterium *Pseudomonas syringae* pathovar *aesculi*, has now been reported from numerous other countries (e.g., Belgium, France, Germany, Ireland, Netherlands and United Kingdom). During a recent survey, molecular analysis and pathogenicity testing identified bleeding canker from horse chestnut (*Aesculus hippocastanum*) in the Czech Republic. This is the first report of the pathogen from this country.

P. syringae pv. *aesculi* is a destructive plant pathogen that causes a serious bleeding canker disease of *Aesculus* species in some parts of Europe. Means of natural spread is unknown; the primary pathway for long-distance spread is with plants for planting. This report expands on the known distribution of this species in Europe. *P. syringae* pv. *aesculi* has not been reported from North America and is considered a quarantine pest for Canada.

SOURCES:

European and Mediterranean Plant Protection Organization, 2009. *Pseudomonas syringae* pv. *aesculi*. EPPO Alert List.

Mertelik, J., Kloudova, K., Pankova, I., Krejzar, V. and V. Kudela, 2013. Occurrence of horse chestnut bleeding canker caused by *Pseudomonas syringae* pv. *aesculi* in the Czech Republic. Forest Pathology 43(2) 165-167.

6 New Method: Detection and identification of Karnal bunt

Current diagnostics procedures for positive confirmation of Karnal bunt are time consuming, challenging, and require a high level of expertise. The current diagnostic protocol involves morphological identification of spores followed by germination of the spores and molecular testing. PCR based tests have been developed and are widely used for identification; however they require mycelium from germinated spores. An enhanced molecular test not requiring spore germination has been developed. The test involves real time PCR based on the ITS region to distinguish *T. indica* from morphologically similar species (i.e., *T. walkeri*, *T. horrida* *T. ehrhartae* and *T. tritici*).

Karnal bunt (caused by *Tilletia indica*) is a floret-infecting fungal pathogen of wheat and triticale that can reduce grain quality. *Tilletia indica* is not known to occur in Canada and is regulated as a quarantine pest. The protocol described here may be of interest to diagnosticians or plant pathologists responsible for screening domestic or imported seed or grain samples for *Tilletia* species.

SOURCE:

Tan, M., Brennan, J.P.,Wright, D. and G. M. Murray, 2013. A review of the methodology to detect and identify Karnal bunt—a serious biosecurity threat. Australasian Plant Pathology Volume 42(1):95-102.

Botany

7 New Report: Capeweed seeds in dried flowers

In a recent news article (Harris 2013), capeweed (*Arctotheca calendula* (L.) Levyns) was reported as intercepted as seeds associated with dried flowers from Australia at a port facility in Oakland, California (Harris 2013). This species is a Federal Noxious Weed in the U.S. It is also listed as an A list noxious weed in California where it is established and considered highly invasive. Capeweed is a weed of roadsides, old fields and other disturbed habitats in California (FNA 1993+). It is native to South Africa and Lesotho (USDA-ARS 2013). In addition to California, capeweed is considered naturalized on Norfolk Island and in Australia, Europe, the Azores, and New Zealand (University of Queensland 2011). The species has not been reported in Canada (Brouillet 2010+; CFIA 2011) nor is there any evidence that it is being grown here in cultivation (CFIA 2009).

This is a member of the aster/daisy/dandelion family (Asteraceae) with yellow, daisy-like flower heads. The species is a weed of disturbance in Australia where it is very common as a weed of gardens, lawns and golf courses. It is also a serious agricultural weed in cropland and pastures. Capeweed is also increasingly recognized as an environmental weed in temperate Australia, particularly in coastal areas and in semi-arid and arid regions. It is also a significant problem in the rangelands of southern Australia, where it replaces more palatable native species, especially in areas that are overgrazed. In more natural habitats, capeweed poses a threat to the integrity of plant communities and the survival of threatened species on conservation lands (University of Queensland 2011).

Capeweed is not present in Canada (Brouillet et al. 2010+; CFIA and CFS 2011), nor could any evidence be found that it is imported for planting as an ornamental (CFIA 2009). A cursory check suggests that capeweed is adapted to Mediterranean climates and grows to about NAPPFAST Plant Hardiness Zone 8. There could be suitable habitat and climate in coastal British Columbia and, possibly, southern Nova Scotia.

SOURCES:

Brouillet, L., Coursol, F., Favreau, M. and M. Anions, 2010+. VASCAN, the database vascular plants of Canada.

CFIA, 2009. Cultivated Plants in Canada database (internal database). Canadian Food Inspection Agency and Canadian Ornamental Plants Foundation, Ottawa, ON.

CFIA and CFS, 2011. Plants of Canada Database. Canadian Food Inspection Agency and Canadian Forest Service.

FNA. 1993+. Flora of North America North of Mexico. eFloras.org.

Harris, H. 2013. Customs agents discover invasive weed seeds in shipment at Port of Oakland. Oakland Tribune. Bay Area News Group, Oakland, CA.

University of Queensland, 2011. Fact Sheet: Capeweed, *Arctotheca calendula*, Weeds of Australia, Biosecurity Queensland Edition. Queensland Government.

8 Update: Biology of kudzu

Kudzu *(Pueraria montana* var. *lobata)* is a perennial climbing vine native to temperate and tropical Asia. Kudzu was introduced to North America in the late 1800s as an ornamental shade plant. However, due to the rich crude protein content, digestibility and productivity, kudzu's role quickly changed to that of pasture, fodder and hay by the early 1900s. Unfortunately, this rapidly growing invasive plant is capable of completely replacing existing vegetation. By smothering orchards and plantations, it can result in significant economic losses. Similarly, measures used to control kudzu on power lines, railways and in national parks are costly. Dense populations of kudzu shade out native vegetation and reduce biodiversity. Kudzu is not currently regulated in Canada and its presence is limited to a single region in Southern Ontario. However, the adaptive physiological properties of kudzu and warmer temperatures due to climate change may allow the geographic potential of this species to increase. Lindgren *et al.* (2013) provide a description of the taxonomy and morphology of kudzu. Current and potential distribution are described using habitat and climate models. Also included in this paper is a comprehensive investigation into the habitat, growth and development, reproduction, and population

dynamics of kudzu. Kudzu's response to herbicides, human manipulations, herbivory, diseases and parasites are examined as well. This is a species of concern to Canada due to its potential to cause significant economic and environmental impacts.

SOURCE:
Lindgren, C.J., K.L. Castro, H.A. Coiner, R.E.Nurse and S.J Darbyshire. 2013. The Biology of Invasive Alien Plants in Canada. 12. *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Predeep. Canadian Journal of Plant Science **93**:71-95.

Entomology

9 Introduction: Initial colonization by *Adelges tsugae* (hemlock woolly adelgid)

Introduced from Japan to British Columbia and eastern North America, the hemlock woolly adelgid (HWA) attacks and kills *Tsuga canadensis* (eastern hemlock) and *T. caroliniana* (Carolina hemlock). Long distance dispersal occurs through movement of infested host trees, wind and transport by birds, deer and other mammals. A recent study quantified the relationship between initial colony size and establishment success of HWA a parthenogenetic organism, capable of producing viable offspring without fertilization by a male. Results showed that the number of settled individuals increased with increasing initial ovisac (containing the eggs) density. The study also demonstrate successful establishment of HWA at the lowest initial density of 1 ovisac per tree (although there was high variability at this initial density).

The initial colonization of an area by an invasive organism is sometimes affected by what is called the "Allee effect" where the population growth is reduced at low density. For example, small founder populations may have greater difficulty establishing because of low mate density. All other factors being equal, parthenogenetic organisms like HWA should logically be able to establish in a new, non-infested area more easily than non-parthenogenetic organisms. This research shows that a new population of HWA can become established from a single ovisac.

Hemlock woolly adelgid is a regulated pest for Canada, and regulated areas have been established in British Columbia, Canada where this pest is present.

SOURCE:
Tobin, P., Turcotte, R. and D. Snider, 2013. When one is not necessarily a lonely number: initial colonization dynamics of *Adelges tsugae* on eastern hemlock, *Tsuga canadensis*. Biological Invasions: 1-8.

10 Update: Potential for Spread of Kudzu Bug, *Megacopta cribraria*, into Kudzu-Free Areas

For the first time, first generation kudzu bugs have been successfully reared from egg to adult stage on soybeans (*Glycine max*). Previous reports indicated that only kudzu plant can support this generation. The authors imply that it should now be an accepted fact that this pest may be able to expand its geographical range into other areas of North America (including Canada), where the kudzu plant is absent (or not widely distributed), but spring soybeans are widely cultivated.

Native to Asia, kudzu bug has not been reported in Canada, and is not considered a quarantine pest. Like the brown marmorated stink bug, *Halyomorpha halys*, kudzu bug has the potential to arrive here as a hitchhiker. A report published in the CFIA Plant Science Scan, Edition 2, August 2012, suggested that the kudzu bug, *Megacopta cribraria*, may eventually arrive in Canada from the south-eastern United States by hitchhiking or by travelling on active weather fronts. After arriving, it has the potential to become a significant field pest of legumes. Adult bugs emerging in early spring are the primary means of dispersal into new areas. The August 2012 report indicated that these bugs are obligately dependent on the kudzu plant, *Puereria montana*, for survival leading to speculation that the restricted distribution of the kudzu plant to the south-eastern US, and the obligate dependency of founder populations of the kudzu bug on this plant may be a barrier to the potential for this insect to become successfully established in kudzu-free areas of North America.

This latest report confirms the possibility of founder populations of kudzu bug establishing on soybean crops, making kudzu bug a more important potential

pest that previously reported.

SOURCES:

Del Pozo-Valdivia, A. I. and D. D. Reisig, 2013. First-Generation *Megacopta cribraria* (Hemiptera: Plataspidae) can develop on soybeans. Journal of Economic Entomology, 106(2): 533-535.

CFIA Plant Science Scan, 2012. New Pest: Kudzu Bug, *Megacopta cribraria*, a potential invasive pest of leguminous crops. 2nd Edition, August 2012.

Biotechnology

11 New Application: DNA marker analysis in risk assessment

A recent study by Kavanagh *el al.* (2013) demonstrated the potential utility of DNA marker analysis to identify interspecific gene flow, the transfer of genetic material through interbreeding between sexually compatible plants. Identifying potential gene flow events is a critical component in the risk assessment of Plants with Novel Traits (PNT's), and the detection of hybrids typically involves the examination of phenotypic characteristics. This study included field-based (natural pollination) studies to identify interspecific gene flow between triticale and two related wheat crops (spring wheat and durum wheat). Simple sequence repeat (SSR) markers were selected as genetic markers to assess and quantify hybridization in addition to traditional screening methods examining phenotypic markers. In total, 14 hybrids were identified from 1.9 million screened seeds demonstrating the occurrence of interspecific hybrids is below international thresholds for low level presence. Of these, 11 hybrids were identified using molecular screening, while the morphological screen identified 5 hybrids (2 seeds confirmed as hybrid from both screening tools). The results of this study demonstrate that DNA markers provide a potentially useful screening tool to identify hydridization between PNT's and their relatives.

In conducting Environmental Safety Assessment of Plants with Novel Traits the consequences of gene flow to sexually compatible plants is one of the five key criteria evaluated. Included in the assessment is the likelihood and impact of introducing the novel trait to a wild population and the resulting consequences on reproductive fitness, acquiring a selective advantage, and the establishment and spread of wild relatives.

SOURCE:

Kavanagh, V.B., M.J. Hills, A. Goyal, H.S. Randhawa, A.K. Topinka, F. Eudes and L.M. Hall. 2013. Molecular markers as a complementary tool in risk assessments: quantifying interspecific gene flow from triticale to spring wheat and durum wheat. Transgenic Research [Epub ahead of print].

12 Gene Regulation: Epigenetic control to fruit ripening in tomato

A recent study by Zhong *et. al,* (2013) examined epigenetic changes that occur in tomato as a potential regulatory mechanism involved in the fruit ripening. Tomato is an important model plant for studying fleshy fruit in particular the critically important process of fruit ripening which can only occur after seed maturation. This study demonstrated that substantial epigenetic reprogramming occurs throughout development including demethylation of gene-ripening promoters. In addition, inhibition of DNA methylatransferases resulted in premature fruit ripening. While the developmental signals which trigger epigenetic changes remain elusive, this study provides valuable insight into the role of the epigenome in fruit development and maturation. It also highlights the future direction in crop improvement strategies in order to produce more economically profitable commodities.

Studying epigenetic control of developmental processes has the potential to impact the CFIA's risk assessments of PNT's as we continue to further our understanding of gene regulation. Future studies on epigenetics may provide a gateway to novel gene products.

SOURCE:

Zhong, S., Z. Fei, Y.R. Chen, Y. Zheng, M. Huang, J. Vrebalov, R. McQuinn, N. Gapper, B. Liu, J. Xiang, Y. Shao and J.J. Giocannoli. 2013. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nature Biotechnology. **31(2):** 154-159.

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Correction:

The previous edition of the Plant Science Scan referred to an outdated version of NAPPO RSPM 22. The correct reference is:

North American Plant Protection Organization (NAPPO). 2011. 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.