Potential Susceptibility of Canadian Flora to EU2 Lineage of *Phytophthora ramorum*[1]

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Abstract

A total of 33 host species commonly found in eastern (8) and western (25) Canadian landscapes and forest sites were selected for this study. Detached leaves/needles were inoculated with *Phytophthora ramorum* EU2 lineage mycelia which was isolated from stream bait near an infected larch plantation in Scotland, UK. There was a large variation in susceptibility among hosts. Among the non-conifer hosts, Pacific dogwood, manzanita, camellia, rhododendron and salal in western Canada and sumac, yellow birch, wintergreen and white ash in eastern Canada were the most susceptible to *P. ramorum* EU2 lineage. For conifer hosts, both balsam fir and white spruce in the eastern Canada and grand fir, sitka spruce, Douglas- fir and western larch in western Canada were the most affected following the inoculation.

Introduction

Phytophthora ramorum is an oomycete pathogen and a causal agent of a disease commonly referred to as sudden oak death (SOD). The pathogen causes foliar blight and shoot dieback of nursery plants, including *Rhododendron* and *Viburnum*. It is also responsible for the widespread mortality of tanoak and coast live oak in coastal California and southwestern Oregon, US, as well as, Japanese larch in the UK. There are four distinct clonal lineages of *P. ramorum*, one originally discovered in Europe, but also detected in a few nurseries, waterways, and one wildland site in western North America (EU1), a new lineage recently detected in Europe (EU2), and two lineages only present in North America (NA1 and NA2). The host range of *P. ramorum* is very broad (more than 120 host plants). Many of the host species are currently present in forested and urban areas on the West Coast of the US and Canada. To better assess the risk posed by an exotic pathogen such as *P. ramorum*, it is often a good strategy to evaluate its capacity to infect plants prevalent in the area of interest. This approach has been used with success where potential hosts were identified by artificial inoculation before being found naturally infected by *P. ramorum*. For instance, *Kalmia latifolia* was first identified as highly susceptible to *P. ramorum* under laboratory conditions [(Phytopathology 2002 (92: S81)] and was thereafter found as a host in the UK [(DEFRA. 2003. www.defra.gov.uk/planth/pra/sudd.pdf]. Similar results were published when a larch (*Larix laricina*) was found susceptible for the first time after artificial inoculations in 2008 (Phytopathology 2008 (98: S75)] before Japanese larch (*L. kaempferi*) was reported heavily infected in plantations in the UK in 2009 (Webber and Brasier 2010; Webber and others 2010) (figs. 1 and 2).

In the UK, the current distribution of the EU2 lineage is limited to southwest Scotland and Northern Ireland (NI). The EU2 lineage is almost exclusively the only lineage of *P. ramorum* in NI. The EU2

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lineage has not been found in England and Wales; only the EU1 lineage is present there (fig. 3). The natural hosts for EU2 lineage include Japanese larch, grand fir, noble fir, rhododendron, red oak, *Vaccinium* sp., *Magnolia* sp., and *Pieris* sp. (Personal communication/correspondence with Joan Webber and Alexandra Schlenzig, May 17, 2016 and May 16, 2016, respectively).

To date, we have investigated the susceptibility of selected eastern and western Canadian host plants to three lineages (NA1, NA2, and EU1) [(Elliott and others 2011; Jinek and others 2011). However, there is an urgent need to determine the susceptibility and risk assessment of the newly emerged lineage EU2 in the UK (Van Poucke and others 2012) and its potential threat to Canadian flora and impact on nursery industry and forest ecosystems.

Materials and Methods

Plant Species Tested

Table 1—Western Canada plant species

Host Name	Species
Arbutus	*Arbutus menziesii*
Garry Oak	*Quercus garryana*
Big Leaf Maple	*Acer macrophyllum*
Red Alder	*Alnus rubra*
Poplar	*Populus trichocarpa*
Pacific Dogwood	*Cornus nuttallii*
Paper Birch	*Betula papyrifera*
Camellia	*Camellia japonica*
Salal	*Gaultheria shallon*
Oregon Grape	*Mahonia nervosa*
Rhododendron	*Rhododendron caucasicum x ponticum*
Currant	*Ribes spp.*
Himalayan Blackberry	*Rubus discolor*
Manzanita	*Arctostaphylos spp.*
Bay Laurel	*Umbellularia californica*
Raspberry	*Rubus idaeus*
Blueberry	*Vaccinium corymbosum*
Grapes	*Vitus vinifera*
Western Hemlock	*Tsuga heterophylla*
Lodgepole Pine	*Pinus contorta*
Western Larch	*Larix occidentalis*
Douglas Fir	*Pseudotsuga menziesii*
Sitka Spruce	*Picea sitchensis*
Grand Fir	*Abies grandis*
Western Red Cedar	*Thuja plicata*

Table 2—Eastern Canada plant species

Host Name	Species
Yellow birch	*Betula alleghaniensis*
Sugar maple	*Acer saccharum*
Red oak	*Quercus rubra*
White ash	*Fraxinus americana*
Wintergreen	*Gaultheria procumbens*
Sumac	*Rhus typhina*
Balsam fir	*Abies balsamea*
White spruce	*Picea glauca*

Culture and Detached Leaves/Needles Inoculations

Detached leaves/needles representing eastern and western Canadian regions (tables 1 and 2) were inoculated with mycelia from a single isolate of *P. ramorum* EU2 (PFC5414) acquired under a Canadian Food Inspection Agency (CFIA) Permit #P-2013-03068 from Alexandra Schlenzig (Scottish Government, Agr, Food & Rural Comm. Directorate). The Scottish EU2 isolate was baited from a stream coming from an infected larch plantation in 2012.

Healthy-looking leaves and needles were collected, rinsed in sterile distilled water (twice) and blotted with a paper towel to remove excess moisture prior to wounding and inoculation with an agar plug. Ten leaves were wounded next to the midrib using forceps; whereas, conifer needles were cut at their base with a surface-sterilized scissor. After wounding, a 5 mm plug of *P. ramorum* EU2 lineage inoculum or blank V8 agar plug was placed mycelium side down over the wounded area on the abaxial side of the leaf or on three needles joined together at their base. After 10 days, leaves were photographed on a scanner and lesion size on each leaf caused by *P. ramorum* EU2 lineage was measured using ASSESS software (Lamari 2002) or measured with a ruler for needles. Lesion area was adjusted for lesion caused by wounding in the control (no inoculum) treatments and considered to be zero if the lesion is equal to or less than that caused by wounding.

Results and Discussion

There was a large variation in susceptibility among tested Canadian hosts to infection by the EU2 lineage of *P. ramorum* (tables 3 and 4). Among the non-conifer hosts, Pacific dogwood, manzanita, camellia, rhododendron, and salal in the west (fig. 4), and sumac, yellow birch, wintergreen, and white ash in the east were the most susceptible to *P. ramorum* EU2 (fig. 5). For conifer hosts, we found both balsam fir and white spruce in the east and grand fir, sitka spruce, Douglas-fir, and western larch in the west to be the most susceptible. These results extend the known potential host range for the *P. ramorum* EU2 lineage.

The detached, *in vitro*, leaf/needle inoculation method of Elliott and others 2011was used in the current investigation, as this method and other published SOD research work elsewhere are well established and applied as approved USDA Animal and Plant Health Inspection Service (APHIS)/CFIA protocols for testing *P. ramorum* host ranges throughout North America. The natural hosts for EU2 lineage in the UK include Japanese larch, grand fir, noble fir, rhododendron, red oak, and *Vaccinium* (Webber and others 2014).

The Canadian Forest Service mandate is to monitor the EU2 lineage situation in the UK and to work closely with the CFIA to update the existing Canadian Pest Risk Assessment (PRA) to address new, relevant *P. ramorum* information as it arises. Furthermore, most recently, a draft genome assembly for *P. ramorum* EU2 lineage was collected from outbreak sites in Scotland (Sambles and others 2015). This information will enhance our understanding of the infection biology of the pathogen. Also, will assist researchers worldwide in accelerating our knowledge and relationships between the four lineages of *P. ramorum* (i.e., NA1, NA2, EU1, and EU2), as well as in developing molecular diagnostic assays for detection and field monitoring of the EU2 lineage (Sambles and others 2015; King and others 2015). Ongoing research is focused on the evaluation of sporulation potential of the EU2 lineage of *P. ramorum* on Canadian flora. Our study confirms the potential threat of the EU2 lineage of *P. ramorum* if it becomes established in Canadian nurseries and wildlands. Furthermore, it will have a great impact on the Canadian horticultural industry, biodiversity, and sustainability of forest ecosystems.

Table 3—Leaf necrosis (% lesion ± SE) on western Canada hosts 10 days post-inoculation (n=5). The most susceptible species are shown in bold.

Host	Rep1	Rep2
1-Arbutus	11.7 ± 3.48	4.9 ± 1.53
2-Garry oak	0.7 ± 0.12	0.8 ± 0.12
3-Bigleaf maple	2.3 ± 0.50	3.1 ± 0.52
4-Red alder	2.8 ± 0.83	1.6 ± 0.37
5- Poplar	4.6 ± 1.65	2.4 ± 0.64
6-**Pacific dogwood**	85.7 ± 5.97	81.2 ± 6.06
7-Paper birch	19.4 ± 1.75	15.7 ± 4.18
8**-Camellia**	47.0 ± 14.23	31.4 ± 2.91
9-**Salal**	18.3 ± 2.35	25.1 ± 3.41
10-Oregon grape	1.2 ± 0.31	1.7 ± 0.56
11-Rhododendron	31.6 ± 4.60	20.1 ± 5.68
12-Currant	6.4 ± 1.20	9.3 ± 2.02
13-Himalayan blackberry	0.1 ± 0.05	0.0 ± 0.01
14-**Manzanita**	58.4 ± 8.55	46.9 ± 8.60
15-Bay laurel	3.3 ± 1.08	1.7 ± 0.25
16-Raspberry	10.1 ± 10.16	0.1 ± 0.03
17-Blueberry	6.3 ± 3.78	15.3 ± 2.30
18-Grapes	11.2 ± 6.52	2.3 ± 1.15
19-Western hemlock	0.0	5.4 ± 0.00
20-Lodgepole pine	0.0	8.2 ± 3.02
21-**Western larch**	51.4 ± 5.65	86.1 ± 3.26
22-**Douglas fir**	24.7 ± 13.0	4.4 ± 23.04
23-**Sitka spruce**	54.0 ± 4.58	76.6 ± 6.02
24-**Grand fir**	68.9 ± 5.35	71.5 ± 6.82
25-Western redcedar	0.2 ± 0.15	0.6 ± 0.43

Table 4—Leaf necrosis (% lesion ± SE) on eastern Canada hosts 10 days post-inoculation (n=5). The most susceptible species are shown in bold.

Host	Rep1	Rep2
26-**Yellow birch**	93.0 ± 3.81	83.7 ± 6.52
27-Sugar maple	10.6 ± 5.43	2.6 ± 2.11
28-Red oak	14.5 ± 7.55	34.5 ± 14.73
29-**White ash**	34.1 ± 17.52	12.2 ± 11.3
30**-Wintergreen**	83.3 ± 10.42	86.5 ± 10.91
31-**Sumac**	8.2 ± 0.07	41.1 ± 1.67
32-**Balsam fir**	54.0 ± 2.74	57.2 ± 9.41
33-**White spruce**	81.5 ± 0.96	83.2 ± 0.00

Conclusions

1. For non-conifer species, Pacific dogwood, manzanita, camellia, rhododendron, and salal in the west; while yellow birch, wintergreen, sumac, and white ash in the east, were the most susceptible Canadian flora to infection by the EU2 lineage.
2. For conifer hosts, we found both balsam fir and white spruce in the east and grand fir, sitka spruce, Douglas-fir, and western larch in the west to be the most susceptible to EU2 lineage infection.
3. These results extend the known potential natural host range of the EU2 lineage of *P. ramorum* which include Japanese larch, grand fir, noble fir, rhododendron, red oak, *Vaccinium* spp., *Magnolia* sp., and *Pieris* sp. in the UK.
4. Research is ongoing to evaluate the sporulation potential of the EU2 lineage on Canadian flora (i.e., to discover the "spore pump" hosts.
5. The Canadian Forest Service is closely monitoring the situation of the EU2 lineage in the UK and working with the CFIA to update the existing Canadian PRA and address new relevant *P. ramorum* information as it arises.

Acknowledgments

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Figure 1—Symptoms of sudden larch death (SLD) in Japanese larch plantations (Crown dieback). (Courtesy of the Forestry Commission, UK).

Figure 2—Cankers, resin bleed on stem and branches, and necrotic needles of Japanese larch. (Courtesy of the Forestry Commission, UK).

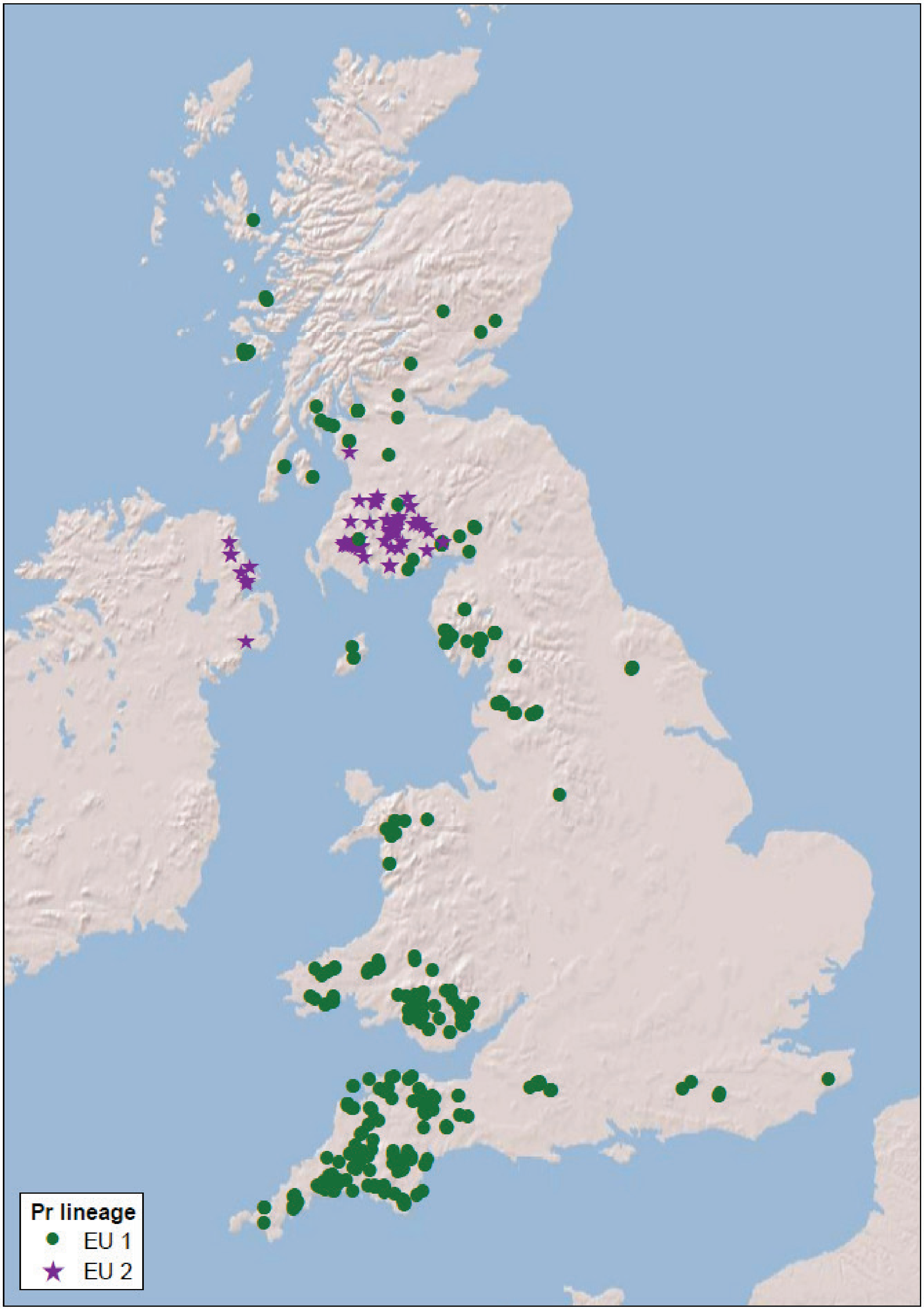

Figure 3—Distribution map of *Phytophthora ramorum* lineages EU1 and EU2 in the UK (Courtesy of Joan Webber, UK Forest Research).

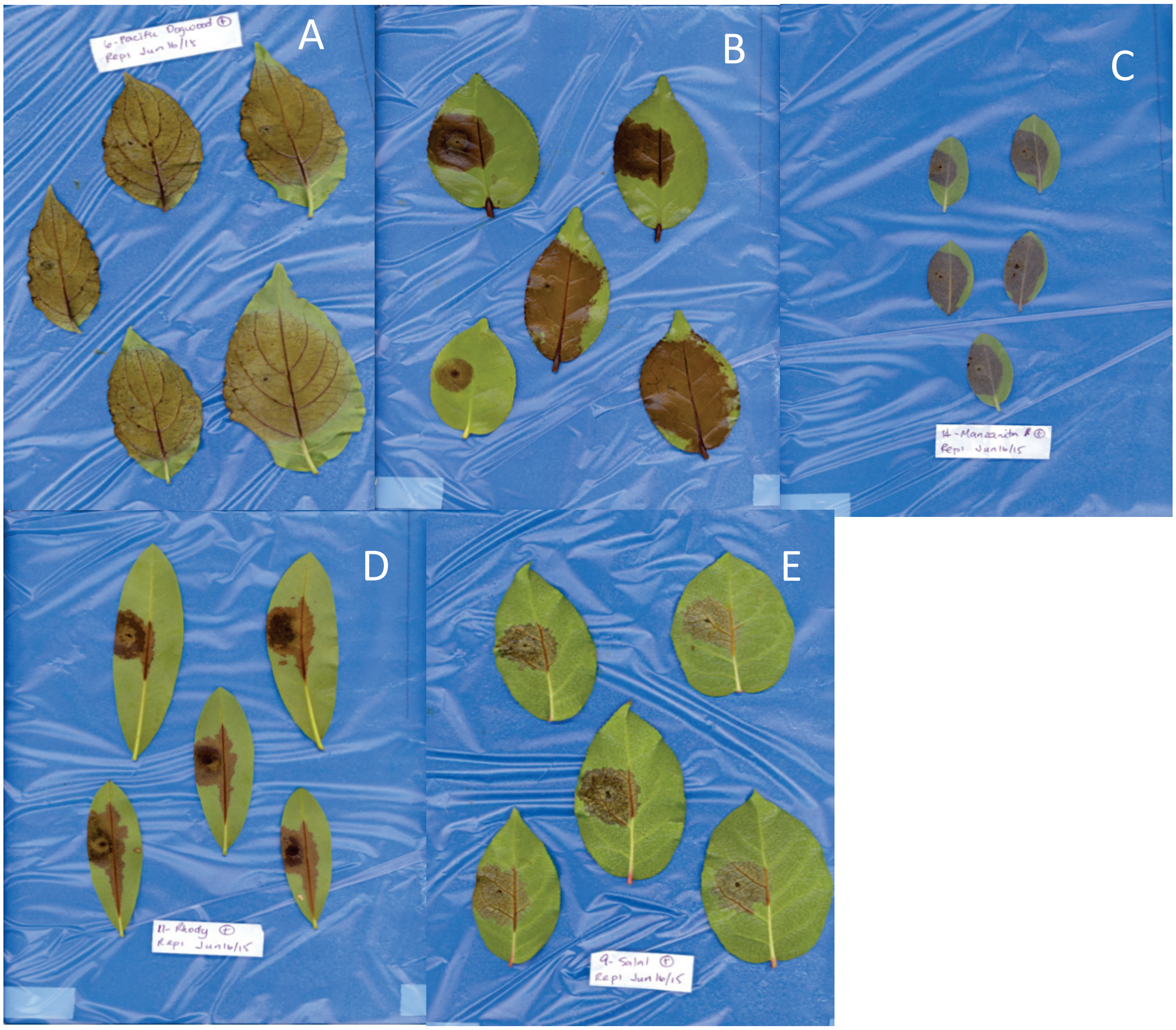

Figure 4—Lesions formed by *P. ramorum* EU2 (PFC5414) on detached leaves of highly susceptible western Canada hosts. A. Pacific dogwood; B. Camellia; C. Manzanita; D. Rhododendron; E. Salal.

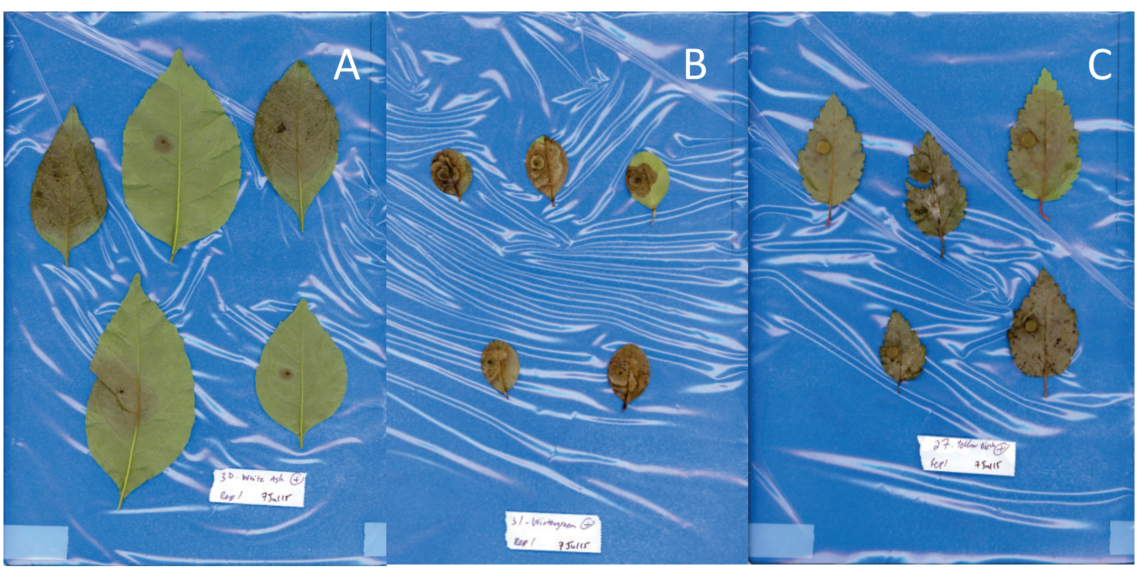

Figure 5—Lesions formed by *P. ramorum* EU2 (PFC5414) on detached leaves of susceptible eastern Canada hosts. A. White ash; B. Wintergreen; C. Yellow birch.