Characterization of bronze leaf disease in western Canadian aspen and poplar trees

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Abstract: Aspen and poplar trees are important horticultural plants grown in Canada for aesthetic, commercial woodlot and windbreak applications. Bronze leaf is a destructive disease in Populus spp. and is caused by the fungal pathogen Apioplagiostoma populi Barr. This pathogen is often difficult to isolate and confirm from infected plant tissues and has been mainly identified by disease symptoms and morphological characteristics of A. populi when fruiting bodies form on infected leaves or branches. Affected leaves and branches typically become necrotic and bronze in colour. Air-borne spores and nursery shipments containing infected plants play an important role in the efficient movement of the pathogen. In this study, bronze leaf disease samples from symptomatic trees in Canada were examined microscopically for A. populi perithecia and asci. Pathogen-specific genomic sequences were identified for the development of sensitive stringent diagnostics that indicated branches and petioles were the most effective tissues for detecting A. populi. Leaf samples from symptomatic trees were collected in Canada and examined for perithecia to microscopically characterize A. populi asci and ascospores. Disease associated DNA sequences of the internal transcribed spacer (ITS) 5.8S region of the nuclear ribosomal, β-tubulin 2, and translation elongation factor-1-γ chain, were isolated from perithecia and symptomatic tree samples. Morphological and molecular biological data from this study characterized the relationship and epidemiology of A. populi and enabled the development of rapid diagnostic methods that restrict the extent of further losses in amenity and commercial plantings of aspen and poplar.

Key words: bronze leaf disease, aspen, poplar, Apioplagiostoma populi, biomarker, management.

Résumé : Le tremble et le peuplier sont d’importantes variétés horticoles cultivées au Canada à la fois comme essences décoratives, pour leur bois ou comme brise-vent. La feuille bronzée est une maladie destructrice qui s’attaque aux espèces du genre Populus et a pour origine le cryptogame Apioplagiostoma populi Barr. Difficile à isoler, la présence du champignon dans les tissus infectés n’est souvent confirmée que par l’apparition des symptômes et la morphologie du cryptogame, quand ses organes fructifères apparaissent sur les feuilles ou les branches touchées. Habituellement, les feuilles atteintes se nécrosent et prennent une couleur bronzée. Les spores, véhiculées dans l’air, et les stocks de pépinière livrés avec des plants contaminés pourraient jouer un rôle important dans la dispersion du champignon. Les auteurs ont examiné au microscope des échantillons de feuilles malades prélevés sur des arbres symptomatiques au Canada pour y déceler les périthèces et les asques du parasite. Après identification des séquences spécifiques au génome de l’agent pathogène en vue du développement de méthodes de diagnostic sensibles et rigoureuses, les auteurs en sont venus à la conclusion que les branches et les pétioles sont les tissus les plus utiles pour dépister A. populi. Cela fait, ils ont échantillonné des feuilles sur des arbres symptomatiques au Canada, puis les ont examinées à la recherche de périthèces afin d’établir les caractéristiques microscopiques des asques et des ascospores. Les séquences d’ADN associées à la maladie dans la région 5.8S de l’espaceur interne de transcription (ITS) du ribosome nucléaire, de la β-tubuline 2 et de la chaîne du facteur 1-γ d’élongation translationnelle ont été isolés dans les périthèces et les échantillons prélevés sur les arbres symptomatiques. Les données morphologiques et celles...
de la biologie moléculaire issues de cette étude ont permis de préciser la généalogie et l'épidémiologie d'A. populi, ainsi que d'élaborer des méthodes de diagnostic rapides qui réduiront les pertes dans les plantations ornementales et commerciales de trembles et de peupliers. [Traduit par la Rédaction]

**Mots-clés :** maladie des feuilles bronzées, tremble, peuplier, Apioplagniostoma populi, biomarqueur, lutte.

### Introduction

Poplar (*Populus* spp.) is commonly used as an ornamental tree, a wind break, and in the pulp and wood industries due to its rapid growth, hardiness and favorable properties of its wood fibre (*DeBell et al.* 2002). Bronze leaf disease (BLD) affects poplar, aspen and their hybrids and is characterized by the infected, dark purplish-brown to bronze-coloured leaves and branches on infected trees (*Kawchuk et al.* 2010; *Ostry et al.* 2012). An infected tree may die within 3–5 yr of initial infection without intensive remedial actions such as pruning and sanitation (*Smith et al.* 2002). The bronze leaf disease caused by *Apioplagniostoma populi* Barr in poplar was first reported in the USA and Canada in 1956 and 2002, respectively (*Cash and Waterman* 1957; *Northover and Desjardins* 2003). Recently, the disease has spread in Canada causing premature mortality of trees such as *Populus x canescens* Smith (Tower poplar), *P. tremula* L. (Swedish columnar aspen), and *P. tremuloides* Michx (Trembling aspen) (*Kawchuk et al.* 2010). Disease symptoms resemble drought injury and include chlorosis and necrosis of leaves with sometimes distinctive green or yellow-coloured veins in infected leaves. Symptoms become severe in late summer, producing dried leaves and subsequent dieback, thus reducing its aesthetic and commercial value (*Dance* 1957; *Sinclair et al.* 1987; *Smith et al.* 2002). Disease diagnosis is often difficult since symptoms resemble those caused by other abiotic and biotic stresses in poplar trees and signs of the pathogen, such as the presence of fruiting bodies on infected tissues, may be hard to recognize or may even be absent.

The BLD found in Canada may, under certain conditions, produce dark brown, beaked perithecia on upper and lower leaf surfaces of infected hybrid poplar leaves (*Northover and Desjardins* 2003). Members of the order Diaporthales produce perithecial fruiting bodies on overwintered leaves and stems of herbaceous plants and include a number of plant pathogenic fungi such as *Cryphonectria parasitica*, the chestnut blight fungus (*Walker et al.* 2014). Ascomycetes produce ascospores resulting from meiosis in asci within the perithecia (*Hawksworth et al.* 1995). Morphological features, such as formation of the peritheciun, ascus structure and the ascospore shape, have been used to classify taxa (*Barr 1978, 1990; Rossmann et al.* 2007). For example, based on the acervular conidiomata on the BLD symptomatic leaves, it has been suggested that a *Discula* spp. may be the anamorph of *A. populi* (*Smith et al.* 2002). Systemic movement of the pathogen has been demonstrated within the host using ascospores as inoculum and BLD was confirmed by the presence of characteristic perithecia (*Sinclair et al.* 1987; *Smith et al.* 2002). Initial studies in Canada showed bronze-coloured symptoms in poplar leaves were often associated with infection by other fungi, such as *Gnomonia* and *Gloeosporium* spp. (*Dance* 1957). However, *Smith et al.* (2002) suggested that the *Gnomonia* spp. found in Canada could actually be *A. populi* due to their similarities in morphology. The variations in morphological characters illustrate a need for additional data to support the accurate identification of *A. populi* and to provide reliable diagnostic assays.

Studies of the Diaporthales inciting destructive diseases provides important information regarding pathogenicity and epidemiology (*Rehner and Uecker* 1994; *Zhang and Blackwell* 2001; *Rossmann et al.* 2007). Characterizing the cause of BLD using molecular diagnostics is becoming increasingly important for the forestry and horticultural industries as symptoms can resemble many other abiotic and biotic stresses. In the present study, we examined morphological characteristics and isolated unique DNA sequences of the Canadian BLD pathogen, *A. populi*, to develop reliable diagnostics for detection and characterization of the pathogen genotypes, to prevent further spread of this destructive horticultural disease through appropriate management strategies, such as the removal and destruction of badly infected trees.

### Materials and Methods

**Isolate collection and DNA extraction**

Leaves, branches and petioles from symptomatic aspen and poplar trees in the western Canadian provinces of Alberta, British Columbia, and Manitoba were collected and surface sterilized with 1% NaOCl, followed by rinsing with sterile water (*Northover and Desjardins* 2003). Trees showing symptoms that were sampled included aspen and poplar from nurseries, urban ornamental plantings, and rural wind breaks. In total, 72 independent, bronze leaf disease-like symptomatic stem and leaf samples from 11 locations were used for the analysis (Table 1). Attempts to culture the pathogen using procedures developed for fungi that are difficult to culture were unsuccessful (*Inglis et al.* 2000; *Ostry et al.* 2016). The symptomatic tissues were preserved and stored at 4 °C for microscopic observations and measurements of presumptive pathogen structures, such as fruiting bodies and spores. Genomic DNA was extracted following the cetyltrimethylammonium bromide (CTAB) total DNA isolation method of *Doyle and Doyle* (1990) from the plant samples.
Table 1. Distribution of samples between 2009 and 2021 exhibiting bronze leaf disease (BLD)-like symptoms, and detection of Apioplagniostoma populi internal transcribed spacer (ITS) 5.8S region of the nuclear ribosomal DNA sequence.

| Location          | No. of tree samples\(^a\) | Tissue\(^b\) | BLD\(^c\) | Sequence\(^d\) |
|-------------------|---------------------------|--------------|----------|---------------|
| Abbotsford BC     | 3                         | SA           | —        | —             |
| Brandon MB        | 4                         | SA, TP       | —        | —             |
| Brooks AB         | 10                        | SA, TP       | 5        | A, B          |
| Calgary AB        | 10                        | SA           | 2        | B             |
| Camrose AB        | 8                         | SA           | 2        | C             |
| Carman MB         | 4                         | TP           | 4        | D             |
| Edmonton AB       | 3                         | SA           | —        | —             |
| Grande Prairie AB | 1                         | SA           | 1        | C             |
| Lethbridge AB     | 18                        | SA, TA, TP   | 6        | A, B          |
| Morden MB         | 6                         | SA, TP       | 3        | D             |
| Winnipeg MB       | 5                         | SA           | —        | —             |

\(^a\)Number of tree samples from different locations in the community.
\(^b\)Tissues include Swedish columnar aspen (SA), trembling aspen (TA), and tower poplar (TP) and for each tree PCR was completed on a leaf, branch and petiole sample.
\(^c\)Number of positive amplifications of the bronze leaf disease pathogen A. populi using nrDNA ITS primers.
\(^d\)Sequence genotype of the bronze leaf disease pathogen A. populi nrDNA ITS.

Designing primers and PCR

Target rDNA ITS sequence was initially amplified with the polymerase chain reaction (PCR) using oligonucleotides to the universal sequences ITS4 and ITS5 for developing A. populi specific oligonucleotides (Schoch et al. 2012, White et al. 1990). Sequences of the β-tubulin 2 (Tub2) and translation elongation factor-1-γ chain (EF1) were amplified with primers ApTub2f 5’AGAACACAAACGACATCCGGCTATCTT3’ and ApTub2r 5’CAATCCGGAAAGACAGTATCTTTGATTTTTCA3’ or ApEFf 5’GCCTACGGTGCTTGGCGAACACTCA3’ and ApEff 5’ATACATGGGATGCGCCAATCCGAC3’ derived from conserved terminal coding sequences. PCR conditions include use of 25 μl with 1 μl genomic DNA, 0.1 unit KAPA HiFi DNA Polymerase (KAPA BioSystems), 5 μl 5X Fidelity buffer, 2.5 mmol·L\(^{-1}\) dNTPs, 2.5 mmol·L\(^{-1}\) MgCl\(_2\), and 1.0 mmol·L\(^{-1}\) of each primer. Amplification was performed at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min. The final extension was 72 °C for 10 min. The PCR products were separated in 1% Tris-acetate-EDTA (TAE) agarose gels stained with ethidium bromide, ligated into pGEM-T Easy vector (Promega) and sequenced. PCR-negative controls consisted of asymptomatic columnar and trembling aspen collected from BLD-free areas in Alberta and British Columbia, burr oak (Quercus macrocarpa), and deionized H\(_2\)O. Sequences of Tub2 and EF1 were amplified with the SuperScript IV One-Step RT-PCR kit (Invitrogen) from A. populi perithecia RNA extracted using a Total RNA kit (Norgen Biotek).

Sequences were used for designing highly specific oligonucleotides ApITsf 5’GCTGGAACAGAAACGGCCTCGCCG 3’ and ApITsr 5’GGGAGAGATTTTACGGCCAGAATGC3’ for stringent detection of the A. populi rDNA ITS based on available sequences. The primers were tested for their specificity using the program GeneRunner (Hastings Software, Hastings, NY, USA) and blasting against the GenBank non-redundant nucleotide database. Specific primers were tested with isolates of Ceratocystis fimbriata (ATCC 24096), Fusarium oxysporum (ATCC MYA-4834), Phytophthora ramorum (ATCC >MYA-3676), and Verticillium dahliae (AAFC LRC-1203) to confirm specificity and show that non-target DNA of pathogens and saprophytes that commonly occur in western Canada, would unlikely be amplified. PCR was done in 25 μl with 1 μl genomic DNA, 0.1 unit KAPA HiFi DNA Polymerase, 5 μl 5X Fidelity buffer, 2.5 mmol·L\(^{-1}\) dNTPs, 2.5 mmol·L\(^{-1}\) MgCl\(_2\), and 1.0 mmol·L\(^{-1}\) of each primer. Amplification was performed at 94 °C for 5 min followed by 25 cycles of 94 °C for 1 min and 72 °C for 1 min. The final extension was 72 °C for 10 min. To confirm the identity of the PCR products, the bands were ligated into pGEM-T Easy vector as described above and sequenced. Samples that produced bronze leaf disease-like symptoms but were negative with ApITsf and ApITsr were examined further with the universal ITS4 and ITS5 primers and amplified products cloned and sequenced to confirm the absence of A. populi. In addition, all samples were stored for at least 1 yr in a mesh bag at 4 °C in storage and suspended outside to assist in the production of A. Populi acervuli or peritheca.
Sequence and phylogenetic analysis

Sequences were determined in forward and reverse directions for at least two clones from each sample. The ITS and 5.8S rDNA nucleotide sequences (GenBank KP637024, OK356802, OK356803, OK356804), β-tubulin (OK501149), and translation elongation factor-1-γ (OK501150) were blasted against the GenBank non-redundant nucleotide database to find homologous fungal sequences. Sequences were aligned with MultiAlin to identify polymorphisms for diagnostics and relationships (Corpet 1988). Selected GenBank 18S rDNA, ITS1, 5.8S rDNA, ITS2, and 28S rDNA sequences were aligned with CLUSTALW (Larkin et al. 2007) and a rooted tree was generated using Molecular Evolutionary Genetic Analysis (MEGA) v. 10.15 (http://www.megasoftware.net) to show relationships.

Results

Disease and morphological characteristics of Apioplagiostoma populi Barr

In Canada, the incidence and distribution of foliar symptoms on aspen and poplar trees that are similar to bronze leaf disease is increasing. Samples were recovered from shelterbelts, residential yards, urban parks and nurseries in Alberta, British Columbia, and Manitoba that experienced chronic disease symptoms, but A. populi was only detected in the Alberta and Manitoba samples (Fig. 1). Perithecia emerged from some symptomatic infected leaves (<2%), following storage at 4°C for 2–6 mo and occurred on upper and lower leaf surfaces (Fig. 2). The wall of the perithecium consisted of densely packed, dark brown-walled cell layers, as previously reported (Frohlich and Hyde 1995). Our observations confirmed that the dark brown to black perithecia were 150–200 wide × 100–150 μm long (Fig. 2). Asci produced by the perithecium were fusoid clavate, 30–40 × 10–14 μm with a conspicuous apical ring and contained hyaline, two-celled ascospores 10–14 × 3–10 μm that were ellipsoid clavate with a relatively short basal cell (Fig. 2). The sizes of ascospores reported by Northover and Desjardins (2003) were 10.5 to 14.5 × 2–3 μm and spores from the samples of A. populi we examined were similar in size.

Molecular analysis and diagnostics of Apioplagiostoma populi

Genomic DNA extracted from isolated perithecia for molecular characterization provided initial complete ITS rDNA sequences and identified polymorphisms that were suitable for molecular diagnostics of BLD (GenBank KP637024, OK356802, OK356803, and OK356804). The ITS region of the A. populi sequence was quite different from other similar fungal sequences in the same family (Fig. 3). Phylogenetic relationships based on the 18S rDNA, ITS1, 5.8S rDNA, ITS2 and 28S rDNA showed A. populi forming a clade with Plagiostoma pulchellum (Pp MH864032), and Discula desructiva (Dd MH862245) (Fig. 3B).

The Tub2 and Efi loci provide additional sequences for A. populi loci (GenBank OK501149 and OK501150) that may be used as secondary confirmation for the presence of the pathogen. As expected, the Tub2 locus was relatively conserved at the amino acid level with a higher
Fig. 3. Alignment of Apioplagiostoma populi sequences with closely related fungi. (A) The 18S ITS1 5.8S ITS2 28S nuclear ribosomal DNA nucleotide sequence of A. populi Canada (Apc GenBank KP6370240) and Minnesota (Apm KR081252), Plagiostoma pulchellum (Pp MH864032), and Discala destructiva (Dd MH862245). (B) Phylogenetic relationship using fungal sequences containing part of 18S rDNA, ITS1, 5.8S rDNA, ITS2 and part of 28S rDNA sequences. The sequences were aligned using CLUSTALW in TREX online tool and include A. populi (Apc and Apm), P. pulchellum (Pp), D. destructiva (Dd), Cryphonectria naterciae [Cn (Genbank MH865223)], Greeneria uvicola [Gu (GenBank MN611374)], and Phytophthora ramorum [Pr (GenBank NR147877)]. (C) Alignment of β-tubulin 2 amino acid of A. populi (GenBank OK501149) with Valsa mali (Vm KUI53087) and Plagiostoma petiolphilum (Pp ADY1974). (D) Alignment of translation elongation factor-1-γ chain amino acid sequence of A. populi (GenBank OK501150) with Vals mali (Vm KUI57956) and Madurella mycetomatis (Mm KXX78874).

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level of polymorphism in the degenerate nucleotide sequence was identified in the Tub2 locus that could be used to confirm presence of the BLD pathogen in diseased trees. A higher level of amino acid polymorphism was observed in the EF1γ than the Tub2 locus when compared with other fungi and this is likely a result of the sequence not having been isolated from any of the related Apioplagiostoma and Plagiostoma spp. (Figs. 3C and 3D).

Interestingly, A. populi was not detected in all tissues from infected samples and the pathogen was observed more consistently in petiole and stem tissues rather than leaves, suggesting a systemic association with the vascular tissues as reported by Ostry et al. (2016) and observed with other species in the family Diaporthales (Fig. 4A). Perithecia and conidia of A. populi were only observed in diseased samples that amplified the reported 18S ITS1 5.8S ITS2 28S nuclear ribosomal DNA nucleotide sequence (Table 1). The ITS sequence amplicon obtained from ITS4 and ITS5 primers, used to design stringent sequence specific oligonucleotides for the specific ITS1, 5.8S and ITS2 rDNA regions of A. populi, produced a unique product of 547 bp (Table 1). Results showed that this A. populi ITS sequence was produced with DNA extracted from perithecia and diseased tissues but was not present in asymptomatic plant samples (Fig. 4B).

Nucleic acid extracted from plant tissue infected with A. populi and diluted 1000-fold produced an amplified product with ApITSf and ApITSr primers (Fig. 4B). Amplification of cloned A. populi ITS sequence detected 5 pg of DNA.

Bronze leaf disease pathogen populations
Despite the coding region of A. populi sequence being conserved, the ITS region is highly variable and showed a close relationship only with Plagiostoma spp. and Apioplagiostoma spp. (Fig. 3). The ITS sequence had the closest nucleotide identity to other members of family Gnomoniaceae. Comparison of the A. populi ITS rDNA sequences of the Canadian and United States BLD samples, showed several single nucleotide polymorphisms that differentiate isolates (Fig. 4C). Furthermore, a polyA indel was identified in all four Canadian A. populi genotypes that distinguished the pathogen from the United States variant. Results indicated that the Alberta and Manitoba A. populi isolates were distinct from the Minnesota population and the polymorphism could be useful in tracking movement and evolution of the pathogen.

Discussion
Many members of perithecial ascomycetes in the Diaporthales and Gnomoniaceae are economically important plant pathogens of woody plants (Barr 1978, 1990). For example, C. parasitica (Murill) Barr, which causes chestnut blight, has altered the forest landscape of eastern North America (Anagnostakis 1987). In addition, bitter rot of grape, caused by the asexually reproducing Greeneria uvicola (Berk. & Curt.) Punith., has had a similar impact on grape plantings (Longland and Sutton 2008). The outbreak of bronze leaf disease in western Canada, caused by A. populi, has affected poplar and aspen trees causing economic loss in shelterbelts, nurseries and commercial woodlots (Northover and Desjardins 2003; Kawchuk et al. 2010). Infection typically starts from leaves and branches and eventually becomes systemic, resulting in dieback of branches. Conclusive identification of the disease and causal agent is often challenging and is required to reduce the risk of spreading pathogen to non-infested areas. Currently, there are no known chemical controls for the bronze leaf disease, and early detection of the pathogen would assist efforts to reduce widespread movement and management practices such as pruning and destroying the infected plant materials.

Bronze leaf disease diagnosis has been mainly based on the symptoms, examination of symptomatic leaves in late summer, and the presence of spore-producing fungal structures on infected plant tissues the following spring (Smith et al. 2002; Northover and Desjardins 2003; Kawchuk et al. 2010; Ostry et al. 2012). Distinct morphological characteristics of A. populi include immersed ascomata as blackened dots from the upper and lower leaf surface and the peridium comprising layers of densely packed dark brown walled cells (Frohlich and Hude 1995; Northover and Desjardins 2003). Asci are usually 2–6 spores, saccate or clavate and thin walled. Asciopores are skittle-shaped with a constricted septum, hyaline and smooth walled with a short basal cell. The lateral elongated perithecial beak and the presence of a short basal-celled ascospores have been used to distinguish Apioplagiostoma or Plagiostoma from different genera in the family Gnomoniaceae (Vasilyeva and Stephenson 2010). The sizes of perithecia, asci and ascospores have been reported to range from 150 to 200 × 100 × 150 μm, 26 to 40 × 10 to 14 μm and 10 to 14 × 3 to 6 μm, respectively (Frohlich and Hude 1995; Northover and Desjardins 2003; Kawchuk et al. 2010). In our study, the identified pathogen was most similar to the one reported by Frohlich and Hude (1995).

Bronze-coloured leaves in poplar may occur as the result of various abiotic and biotic stresses (Cash and Waterman 1957; Dance 1957; Hibben et al. 1978; Smith et al. 2002). Although morphological features may distinguish the BLD pathogen from others, to some extent, diagnostics are critical to expedite and confirm the presence of the causal agent of the disease. Isolation of A. populi on artificial media has been challenging and inconsistent (Smith et al. 2002; Northover and Desjardins 2003; Kawchuk et al. 2010; Ostry et al. 2012; Ostry et al. 2016). However, in this study, we were able to develop a molecular biological approach to identify and characterize A. populi in poplar. Sequencing data are limited for this pathogen and were initially reported.
Fig. 4. Tissue specificity and systemic localization of *Apioplagniostoma populi* in infected tissues. (A) Sampling of tissues from various tissues indicated that leaves (white arrow) from infected branches were often negative for *A. populi* (green dot) whereas petioles (yellow arrow) and branches (orange arrow) provided more positive (red dot) results. (B) Agarose gel electrophoresis of the amplified DNA with the stringent diagnostic primers ApITSf and ApITSr, spanning the ITS sequence (550 bp) including the hypervariable ITS1 and ITS2 regions, from *A. populi* (Lane 3) diluted by 10, 100, 1000, 10 000 (Lane 4,5,6,7). A water blank and 100 bp DNA ladder are shown in Lanes 1 and 2, respectively. (C) Identity between *A. populi* samples from Alberta (CNAB) and Manitoba (CNMB) Canada with those from Minnesota (USMN) show several single nucleotide polymorphisms in addition to a Country specific polyA indel in the Canadian variant; CNAB1 KP6370240, CNAB2 OK356802, CNAB3 OK356803, CNMB1 OK356804, USMNE2 KRO81253, USMND2 KRO81252, USMNC KRO81251, USMN3 KRO81250, USMNC16 KRO81249.
for the ITS region of A. populi (GU205341) (Kawchuk et al. 2010). Sequences were subsequently reported for the ITS of A. populi from MN disease samples (Fig. 4C; Ostry et al. 2016). Phylogenetic analysis revealed that the A. populi rDNA ITS sequence is distinct but closely related to Apioplagiostoma, Plagiostoma, and Discula spp. (Fig. 3). Sequences of Tub2 and EF1 γ loci from A. populi provide additional markers that may be used to confirm the identity of the Apioplagiostoma species and further examine relationships and epidemiology (Hill et al. 2011).

Analysis of the various diseased plant tissues in our study showed that the petioles and stems were a better source of pathogen DNA for diagnostics (Fig. 4A). Members of the Diaporthales are known to accumulate their mycelia in vascular tissues and our results suggest that A. populi mycelia also grows systemically in specific tissues that remain viable and influences sampling for diagnostic testing (Ostry et al. 2016). Oligonucleotides designed for the stringent rapid amplification of the 18S nuclear small ribosomal 3’ DNA, ITS1, 5.8S rDNA, ITS2, and 28S large nuclear ribosomal 5’ DNA sequences of A. populi produced a 547 bp PCR product that contained several diagnostic polymorphisms and indels (Figs. 4B and 4C). Alignment of Canadian with United States A. populi ITS rDNA sequences identified country- and region-specific polymorphisms that facilitate determining origin and movement of the pathogen. This diagnostic will increase the sensitivity and reliability in detecting the causal agent of the bronze leaf disease in western Canada and assist with disease management strategies, such as the removal of infected trees and branches, and for monitoring the spread of the disease to other areas. Specificity of this diagnostic was confirmed with no amplification of several other tree pathogens that are found in western Canada. It is recommended that the amplified DNA product is sequenced, especially if this PCR test is used in other areas outside of western Canada.

Bronze leaf disease has become an invasive tree disease in Canada, causing death in ornamental and commercial poplar and aspen species. The morphological and molecular biological tools used in this study to verify and characterise the causal agent A. populi in Canada have assisted in implementing best management practices to prevent rapid disease dispersal within provinces and potentially across the country and thereby help to reduce further disease losses. Reducing long distance movement of pathogens on greenhouse and nursery plants should also deter further dissemination of A. populi (Kalischuk et al. 2012). Additional studies are underway to develop sensitive quantitative multiplex assays, provide additional information on pathogen epidemiology and diversity, and understand the relationships and the fungal species associated with the bronze leaf disease for the development and introduction of both proactive and reactive A. populi control measures.

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