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Development of a Rapid Loop-Mediated Isothermal Amplification Assay for the Detection of *Dothistroma septosporum*

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Abstract: A Loop-Mediated Isothermal Amplification (LAMP) assay was developed for the detection of the pine pathogen *Dothistroma septosporum* (G. Dorog.) M. Morelet. The specificity of the LAMP assay was tested using a selection of pine needle fungi, including *Dothistroma pini* Hulbary, and *Lecanosticta acicola* (Thüm.) Syd.; only *D. septosporum* DNA was amplified by the test. In terms of sensitivity, the assay was able to detect as little as 1 pg of total *D. septosporum* DNA. This assay enables DNA extracted from diseased host needles to be rapidly tested for the presence of *D. septosporum* using relatively simple to operate equipment away from a fully equipped molecular biology laboratory.

Keywords: DNA-based diagnostics; LAMP; *Dothistroma* needle blight

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1. Introduction

Dothistroma needle blight, a disease caused by the pathogens *Dothistroma septosporum* and *Dothistroma pini*, results in significant damage to pines in both natural and plantation settings globally [1]. In British Columbia, the increasing losses due to *D. septosporum* have been linked to climate change [2]. During 2012 and 2013, *D. septosporum* was found on *Pinus contorta* Dougl. var. *latifolia* Engelm., *Pinus banksiana* Lamb., and their hybrid, in northern Alberta, Canada [3]. The identification of *D. septosporum* in Alberta and the potential for eastward spread through the boreal forest have led to research into population genomics [4], host range [3], and, as we describe here, rapid diagnostics for *D. septosporum*, the pathogen associated with the disease in western Canada.

Polymerase chain reaction (PCR)-based diagnostics for detecting *D. septosporum* DNA have been described by Ioos et al. [5], Langrell [6], and Schneider et al. [7]. These diagnostics are sensitive and able to detect the pathogen within infected tissue although they must be performed within a well-equipped laboratory. Loop-Mediated Isothermal Amplification (LAMP) is an alternative to traditional PCR-based methods. LAMP assays use four to six oligonucleotide primers and a DNA polymerase to drive strand displacement DNA synthesis at a constant temperature without thermalcycling [8]. LAMP assays have been developed for point-of-care diagnostics in medicine [9], agriculture [10], and important forest pathogens [11]. Portable instruments such as the Genie® III developed by OptiGene (Horsham, West Sussex, UK) or the BioRanger instrument developed by Diagenetix Inc. (Honolulu, HI, USA) have been developed to allow LAMP assays to be conducted under field conditions.

Here we report the development of a LAMP-based assay to identify *D. septosporum* in culture and to rapidly and accurately detect *D. septosporum* within infected pine needles.

2. Materials and Methods

2.1. Culturing of D. Septosporum

Pure cultures of *D. septosporum* were obtained from infected pine needles by excising a single acervulus and rolling it in a straight line across the surface of a 2% Malt Extract Agar (MEA) plate. Once spore germination was visible under a dissecting microscope (2–3 days at room temperature), a single germinated conidia was transferred to a fresh 2% MEA plate and incubated at room temperature. Alternatively, *D. septosporum* was isolated by surface sterilizing an infected needle in 10% bleach for 5 min to reduce surface contamination and then placing the infected needle on 2% water agar at room temperature to induce sporulation. An erupting conidial mass from an acervulus was then placed on fresh 2% water agar and a single hyphal tip was subcultured onto fresh 1% to 3% MEA [3]. Prior to DNA extraction, liquid cultures were prepared by inoculating 50 mL clarified V8 medium with mycelial plugs from actively growing cultures, then incubating these at 20 °C with occasional shaking for 6–8 weeks. V8 juice amended with 1.42 g $CaCO_3$ per 100 mL was clarified first by centrifugation for 15 min at 2500 rpm and then filtering the remaining supernatant 3 times through Whatman® qualitative filter paper, grade 4. To obtain the clarified V8 growth medium, clarified V8 juice was mixed with dH_2O in a 1:4 ratio.

Pure cultures of *D. septosporum* were obtained from infected *Pinus attenuata* Lemmon, *Pinus attenuata* × *Pinus radiata* D. Don, *P. banksiana*, *P. contorta* var. *latifolia*, *P. contorta* var. *latifolia* × *P. banksiana*, *Pinus monticola* Dougl. ex D. Don, and *P. radiata*, collected from Alaska, Alberta, British Columbia, California, Oregon, and New Zealand. Pure cultures of *D. pini* were obtained from infected *Pinus mugo* Turra, *Pinus nigra* Arnold, and *Pinus ponderosa* P. Laws. ex C. Laws. collected from Iowa, Montana, Nebraska, North Dakota, Ontario, and South Dakota. Pure cultures of *L. acicola* were obtained from infected *P. banksiana*, *P. contorta* var. *latifolia*, and *P. contorta* var. latifolia × *P. banksiana* collected from Alberta and British Columbia. Cultures of *Ceuthospora* sp., *Cyclaneusma minus* (Butin) DiCosmo, Peredo and Minter "simile", *C. minus* "verum", *D. pini*, *D. septosporum*, *Lophodermium conigenum* (Brunaud) Hilitzer, *Pestalotiopsis* sp., *Phytophthora pluvialis* Reeser, Sutton and E. Hanson, and *Strasseria geniculata* (Berk. and Broome) Höhn. were also used (Table S1).

2.2. Extraction of DNA from Cultures

In Canada, two DNA extraction methods were used to extract DNA from fungal cultures. The Qiagen DNeasy PowerPlant Pro kit (Qiagen, Valencia, CA, USA) was used to isolate DNA from mycelium harvested from liquid V8 cultures. Freeze dried mycelium was first ground with one 3 mm stainless steel bead in a SPEX® Mixer/Mill (SamplePrep, Metuchen, NJ, USA) for 30 s at setting 1/30. DNA was then extracted from the ground mycelium following the manufacturer's protocol with the following modifications: the optional phenolic separation solution was added at the fist extraction step and sample disruption by bead beating was replaced by a 10 min incubation at 65 °C. Alternatively, a simple Tris-EDTA extraction method was used to extract DNA from cultures growing on MEA. A single plug (5 mm diameter) was placed in microcentrifuge tubes with 400 µL of Tris-EDTA (10 mM Tris-1 mM EDTA, pH 8.0) solution, one 4 mm and three 2.8 mm stainless steel beads, and placed in a SPEX® 1600 MiniG® (SamplePrep, Metuchen, NJ, USA) bead beater for 3 min at 1500 rpm to disrupt the mycelium. After disruption the sample was centrifuged at 16,000× *g* for 3 min and the supernatant transferred to a new tube. DNA extraction method for all specimens is specified in Table S1. In either case, DNA stock solutions were diluted 100-fold in sterile PCR-grade water to serve as a template for DNA amplification.

In New Zealand, DNA from cultures of *Ceuthospora* sp., *C. minus* "simile", *C. minus* "verum", *D. pini*, *D. septosporum*, *L. conigenum*, *Pestalotiopsis* sp., *P. pluvialis*, and *S. geniculata*

was extracted using the FastDNA kit (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol. DNA of other *Phytophthora* spp. was provided by collaborators and the extraction method is unknown (Table S1).

2.3. ITS-Based Identification of Cultures

The nuclear ribosomal internal transcribed spacer (ITS) was PCR amplified using the total DNA extracted from putative isolates of *D. septosporum* and other fungi isolated from pine needles using the primers ITS-1F [12] and ITS-4 [13] following the protocol described in Feau et al. [3]. The PCR product was purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA), and sequenced by Macrogen (Seoul, Korea) or CHU de Québec—Université Laval Research Centre (Quebec, QC, Canada). To identify fungal cultures, ITS sequences were queried against the NCBI nr database using the BLASTn algorithm [14].

2.4. Extraction of DNA from Needles

DNA was extracted from pine needles using DNAzol® Direct (Molecular Research Centre, Inc., Cincinnati, OH, USA). Needles putatively infected by *D. septosporum*, as indicated by microscopic examination of conidia produced on needles that had been placed at room temperature in a sealed Petri plate with a moist paper towel to induce sporulation, as well as visually healthy needles, were selected for DNA extraction. Needles were either fresh and stored at 4 °C or dried and stored at −20 °C. When fresh, one to two infected needles, cut into 5 mm sections, were placed in a 1.5 or 2.0 mL centrifuge tube, with 100 µL of DNAzol® Direct. The needles were then ground with a SPEX® 1600 MiniG® (SamplePrep, Metuchen, NJ, USA) bead beater using one 4 mm and three 2.8 mm stainless steel balls at 1500 rpm for 3 min. When the needles were dry, one or two needles were ground using the bead beater prior to adding DNAzol® Direct. The DNAzol® and ground needle mixtures were incubated at room temperature for 15 min with occasional inversion. The tubes were then centrifuged at 16,000× *g* for 3 min to pellet debris, the DNA-containing supernatant was transferred to a fresh tube and a 100-fold dilution in sterile PCR-grade water was prepared to serve as template for DNA amplification. The same procedure was used to extract DNA from needles of *P. contorta* var. *latifolia* that were infected with *Elytroderma deformans* (Weir) Darker, *Davisomycella ampla* (Davis) Darker, *Lophodermella concolor* (Dearn.) Darker, *Lophodermium seditiosum* Minter, Staley and Millar, and *Lecanosticta acicola* (Table S2).

2.5. Development of LAMP Primers

The β-tubulin 2 gene was selected as a target for our LAMP assay as it has previously been shown to distinguish *D. septosporum* from close relatives [5,7]. A consensus of partial *D. septosporum* β-tubulin 2 gene sequences was generated from an alignment of GenBank accessions AY808205–AY808230 [15], assembled by Barnes et al. [15], that is broadly representative of *D. septosporum* diversity. The consensus sequence was then compared to the corresponding portions of the β-tubulin sequence data from *L. acicola* (AY808237, AY808238) [15] and *D. pini* (AY808231–AY808236) [15] to identify regions specific to *D. septosporum* and would therefore make suitable targets for the LAMP primers. LAMP primers for the *D. septosporum*-specific regions were developed using default parameters in the LAMP Designer 1.4 software package (PREMIER Biosoft, OptiGene, Horsham, West Sussex, UK). The top ranked primer set, as determined by the software, that distinguished *D. septosporum* and *D. pini* was selected (Table 1; Figure 1). The specificity of the F3/B3 primer pair and the component sequences of the FIP and BIP primers (F1c and F2, and B1c and B2, respectively (Figure 1)) were assessed using Primer-BLAST [16], with default parameters and the nr database for fungi.

Table 1. LAMP primers specific to the β-tubulin 2 gene of *Dothistroma septosporum*.

Primer Name	Sequence 5′-3′
F3	CAGGACAGTATGTGGAATCC
B3	TCGGTGCTTGCCTAGATA
FIP	GACACTCAGTCGCTCTCGCGCGTCATGCAGATTCGTA
BIP	AGGTAGGTGCTCCTCTCCGCAGGATGACGATGTGCTG
LoopF	TGCAGTGCCTTCGTATCTG
LoopB	CCAGTGCTTCAACACTATGC

Figure 1. Position of LAMP primers based on consensus sequence of *D. septosporum* β-tubulin 2 gene sequences retrieved from GenBank (AY808205–AY808230). Primer FIP is a combination of F1c and F2, while primer BIP is a combination of B1c and B2. Differences from *D. pini* are noted by * symbol.

2.6. Preparation of the LAMP Reaction

Primers were first hydrated to a stock concentration of 500 µM using Tris-EDA diluted 10-fold with sterile PCR-grade water. A working stock of primer mix was then prepared that contained 0.8333 µM F3, 0.8333 µM B3, 1.666 µM LoopF, 1.666 µM LoopB, 3.333 µM FIP, and 3.333 µM BIP in PCR-grade water. Primers and template DNA were then combined in Optigene Isothermal master mix (ISO-001) (OptiGene, Horsham, West Sussex, UK) in the proportions of 0.6 master mix, 0.24 primer mix and 0.16 template DNA (i.e., for a 15 µL reaction, use 9 µL isothermal master mix, 3.6 µL of primer mix, and 2.4 µL of template DNA). The final primer concentrations for the LAMP reaction were 0.2 µM F3, 0.2 µM B3, 0.4 µM LoopF, 0.4 µM LoopB, 0.8 µM FIP, and 0.8 µM BIP. Template DNA from cultures or needles consisted of stock DNA diluted 100-fold in sterile PCR-grade water.

Primers, master mix and template DNA were combined with a final volume of 15 µL. The Genie® III instrument (OptiGene, Horsham, West Sussex, UK) was run at 65 °C for up to 60 min, and then annealing ramped from 98 to 80 °C at a rate of −0.05 °C/s for fragment identification. Both positive (pine DNA spiked with 0.1 to 0.01 ng of DNA from *D. septosporum* per 15 µL reaction) and a blank (sterile PCR-grade water) were included in each run. In New Zealand, the Smart-DART (Digenetix Inc., Honolulu, HI, USA) or BioRanger LAMP devices (Digenetix Inc., Honolulu, HI, USA) were used with the same reaction components as were utilized in Canada although the reaction was overlaid with mineral oil as the Smart-DART and BioRanger do not have heated lids.

2.7. Sensitivity of the LAMP Assay

The sensitivity of the LAMP assay was determined using serial dilutions of total *D. septosporum* DNA extracted from three different isolates, all of which were collected from the north Okanagan region of British Columbia from *P. contorta* var. *latifolia*. Total DNA

concentration was first quantified using a Qubit™ 4 Fluorometer (Invitrogen, Waltham, MA, USA) and the Qubit™ dsDNA HS assay kit (Invitrogen, Waltham, MA, USA) and then standardized to 4.17 ng/µL. Serial dilutions were made from 4.17 ng/µL to 4.17 × 10^{-6} ng/µL and 2.4 µL of template DNA was added to each 15 µL LAMP reaction, providing a dilution series from 1 ng to 1.0 × 10^{-6} ng of template DNA in each reaction. The LAMP reaction was run for 60 min.

2.8. Specificity of the LAMP Assay

The specificity of the LAMP assay was assessed using DNA from cultured isolates of *D. septosporum*, *D. pini*, and *Lecanosticta acicola* (Table S1), and using pine needles infected with *L. acicola*, *Lophodermella concolor*, *Lophodermium seditiosum*, and *Elytroderma deformans* (Table S2). In New Zealand, the assay was tested against *Phytophthora* spp. and fungi associated with the foliage of *Pinus radiata* (Table S1). Confirmation of the ability of the LAMP assay to detect *D. septosporum* within infected needles was conducted by using DNA extracted from a subsample of needles from which *D. septosporum* was previously isolated into pure culture and identified by ITS sequencing. In cases where the LAMP reaction was negative, the quality of the DNA was checked by PCR amplifying the ITS region to confirm that the DNA could be amplified.

2.9. Statistical Comparison of the DNA Extraction Methods

The annealing temperature and amplification time for *D. septosporum*-positive DNA samples extracted using the Qiagen DNeasy PowerPlant Pro kit and the simple Tris-EDTA methods were compared using the nonparametric Kruskal–Wallis test as the assumptions of equal variance and normal distribution were not met. The sample from New Zealand was excluded from the analysis as the annealing temperature and amplification time were not recorded. Raw data are provided in Table S1.

3. Results

Our *D. septosporum* LAMP assay has high specificity. DNA extracted from all isolates of *D. septosporum* included in the screening population produced positive amplification while no amplification occurred when the DNA of other fungi or *Phytophthora* spp. that were included in the screening population were used as template. (Table 2). Further, in silico analysis of the LAMP primers using Primer-BLAST suggested that all six primers were specific for *D. septosporum*. Detection times were significantly shorter for DNA samples extracted using the Qiagen method compared to the TE method (H = 44.5345; p = 2.499 × 10^{-11}). In contrast, there was no significant difference between extraction methods in terms of annealing temperatures (H = 1.7184; p = 0.1904) (Table 2). Detection of *D. septosporum* from DNA extracted from infected needles took longer relative to DNA from cultures regardless of method; average detection time was 28.50 (SD ± 9.42) min although detection took as long as 54 min (Table S2). Examples of positive and negative LAMP reactions are presented in Figure 2.

Table 2. Summary LAMP results based on amplification of template DNA extracted from pure cultures and needles. Cultures—Qiagen indicates results for DNA extracted using the Qiagen method and Cultures—TE indicates the results for DNA extracted using the TE method.

Origin and DNA Extraction Method	No. of Positive Amplifications/No. of True Positive Samples	No. of No Amplifications/No. of True Negative Samples	Average Annealing T °C (±SD)	Time (min) (±SD)
Cultures—Qiagen	40/40	55/55	88.57 (±0.13)	15.82 (±1.15)
Cultures—TE	25/25	3/3	88.62 (±0.06)	22.46 (±3.54)
Needles—DNAzol	27/27	5/5	88.55 (±0.14)	28.78 (±9.67)
All	92/92	63/63	88.58 (±0.12)	21.42 (±7.79)

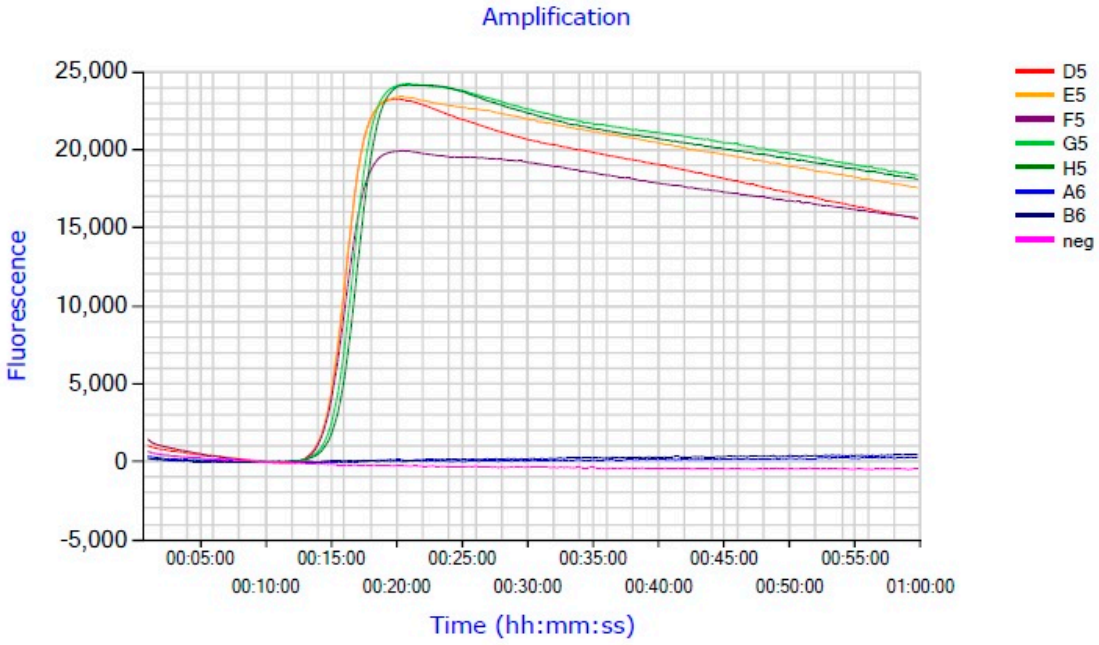

Figure 2. Output from LAMP assay to assess specificity of *D. septosporum* lamp assay. D5 = *D. septosporum* (DSEP-FLNRO-7M-Ne2-TAIGA-591); E5 = *D. septosporum* (DSEP-Whitecourt-Mountain-4077-2-Ne2); F5 = *D. septosporum* (DSEP-EM-Mt-14-Ne1-TAIGA-469); G5 = *D. septosporum* (DSEP-ABTIC-9-Ne1-TAIGA-590); H5 = *D. septosporum* (DSEP-CLG-2-Ne1-TAIGA-614); A6 = *D. pini* (DPIN-Brewer-Lake-Needle1); B6 = *D. pini* (DPIN-Brewer-Lake-Needle2); neg = sterile distilled water.

In sensitivity testing, *D. septosporum* was consistently detected in less than 30 min when at least 2 pg of template DNA was included in the LAMP reaction. The detection limit of our LAMP assay was approximately 1 pg of template DNA (Table 3). Generally, as the concentration of template DNA decreased, the time to detection increased; for example, for 2 pg of template DNA detection took 18–26 min, whereas for 1 ng, detection took 14–16 min (Table 3, Figure 3). The LAMP assay detected the presence of *D. septosporum* within fresh and dried pine needles; however, if needles were damp chambered to induce pathogen sporulation for microscopic examination the LAMP reaction failed.

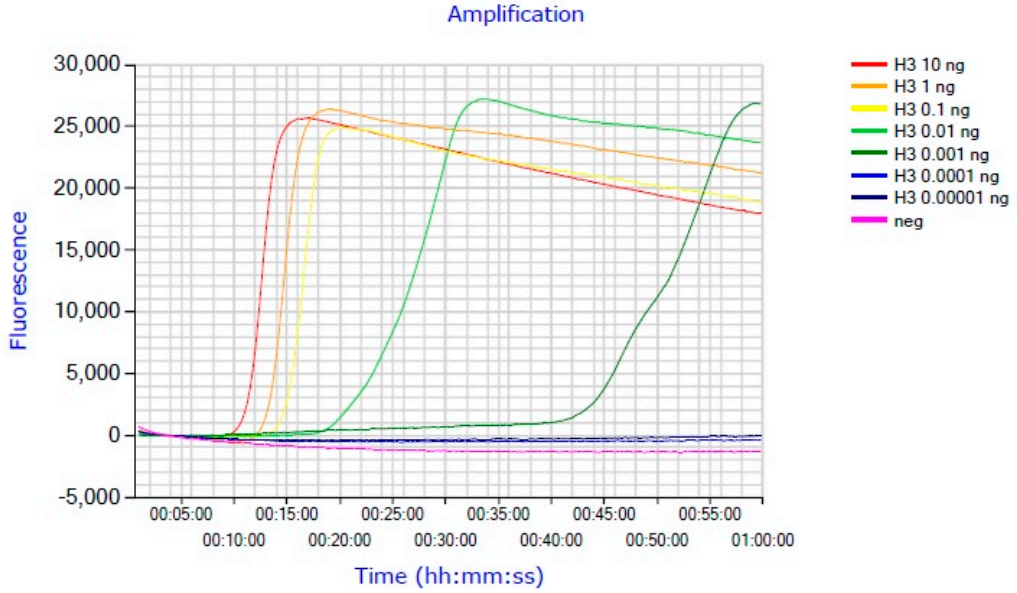

Figure 3. Determining the sensitivity of the LAMP reaction. Isolate H3 = *D. septosporum* (DSEP-Kingfisher-Ne1). Positive results for concentrations from 10 to 0.001 ng/µL. Negative results for 0.0001 ng/µL, 0.00001 ng/µL, and negative control (sterile distilled water).

Table 3. Sensitivity of LAMP assay determined by using serial dilutions of *D. septosporum* DNA.

Sample	ng Template DNA in 15 µL Reaction	Annealing Temp (°C)	Amplification Time (min)
Cherryville Site 2 Tree 2 Needle 2	10	88.67	13.30
	1	88.66	15.45
	0.1	88.73	18.00
	0.01	88.66	25.00
	0.009	88.59	18.00
	0.008	88.54	21.00
	0.007	88.64	21.45
	0.006	88.59	33.15
	0.005	88.57	20.30
	0.004	88.53	23.45
	0.003	88.53	23.45
	0.002	88.54	23.00
	0.001	-	-
	0.0001	-	-
	0.00001	-	-
Mabel Lake Tree 2 Needle 1	10	88.62	13.30
	1	88.62	15.45
	0.1	88.74	16.15
	0.01	88.71	19.30
	0.009	88.53	20.35
	0.008	88.54	25.29
	0.007	88.57	22.55
	0.006	88.68	23.56
	0.005	88.62	26.31
	0.004	88.64	20.19
	0.003	88.58	20.19
	0.002	88.55	25.13
	0.001	-	-
	0.0001	-	-
	0.00001	-	-
Kingfisher Needle 1	10	88.65	12.30
	1	88.67	14.30
	0.1	88.66	16.15
	0.01	88.68	28.00
	0.009	88.52	22.15
	0.008	88.57	21.30
	0.007	88.59	20.30
	0.006	88.62	24.30
	0.005	88.57	20.45
	0.004	88.57	21.30
	0.003	88.60	22.15
	0.002	88.61	18.30
	0.001	88.46	46.30
	0.0001	-	-
	0.00001	-	-

"-" indicates no amplification.

4. Discussion

Dothistroma septosporum, one of the causal agents of *Dothistroma* needle blight, is a pathogen of global importance within both natural and plantation pine stands. This pathogen is morphologically similar to the closely related species *D. pini* [15] and DNA-based assays have been developed to differentiate between these two pathogens [5,7]. As historical distribution records in Canada are based on morphology, it will be necessary to use DNA-based methods to fully understand the geographic distribution of these

pathogens. Development of a LAMP diagnostic assay for *D. septosporum* was prompted by the finding that this species was causing *Dothistroma* needle blight in Alberta [3,4].

The LAMP assay reported here is based on the β-tubulin 2 gene, a locus that has previously been shown to distinguish *D. septosporum* from close relatives [5,7,15]. The results of this study further confirm the suitability of this locus as a diagnostic for *D. septosporum*. The sample population of *D. septosporum* assembled for this study was geographically diverse, with samples representing the different geographic subgroups identified by Capron et al. [4], as well as samples from Alaska, California, Oregon, and New Zealand, suggesting that the assay has wide applicability.

Assessment of a dilution series indicated that the LAMP assay is highly sensitive and able to detect as little as 1 pg of pathogen DNA. The LAMP assay is not as sensitive as Langrell's [6] nested PCR assay that targets the ITS region. Schneider et al. [7] also found their ITS-based assay to be more sensitive than the qPCR assay of Ioos et al. [5] that targets β-tubulin 2. These authors suggested that this difference reflects target copy number; β-tubulin 2 is a single-copy gene whereas ITS has a high copy number [7]. The LAMP assay was as sensitive as the duplex-scorpion qPCR assay of Ioos et al. [5], but not as sensitive as their dual-labelled probe qPCR assay [5], despite both targeting β-tubulin 2. Advantages of the LAMP assay include the relatively easy to use equipment and speed of the assay; however, the assays of Ioos et al. [5] and Schneider et al. [7] can simultaneously detect *D. pini* and *D. septosporum* while the LAMP assay only detects *D. septosporum*.

The two methods used to purify DNA from cultures of *D. septosporum* resulted in significantly different time to detection by the LAMP assay; DNA extracted using the Qiagen method amplified more quickly than DNA extracted directly from mycelial plugs using the Tris–EDTA method. The DNA extracted using the Qiagen method is likely to have been associated with fewer impurities as therefore was a better template for amplification. The final concentration of DNA extracted by the Tris-EDTA method was also likely lower than DNA extracted using the Qiagen method. In contrast, there was no significant difference between the two extraction methods in terms of annealing temperature, indicating that extraction method did not influence specificity. Although the assay worked using DNA extracted with both methods, and is very sensitive, these results highlight the importance of using high-quality DNA, especially in situations where the presence of *D. septosporum* may represent a new distribution record.

The time to pathogen detection was shorter when using DNA extracted from cultures than DNA extracted from infected needles. This is likely due to the higher relative concentration of *D. septosporum* DNA in extracts from cultures; DNA from *D. septosporum* makes up all of the DNA extracted from cultures but only a small proportion of the total DNA when extracted from infected needles. The assay was used successfully to assess the presence of *D. septosporum* if needles were fresh or stored dried at room temperature, at 4 °C, or at −20 °C; however, if needles were damp chambered at room temperature to induce pathogen sporulation, the assay became unreliable. In this case, increases in levels of inhibitory compounds, or a reduction in the relative concentration of *D. septosporum* DNA due to the growth of other fungi might explain this result. Regardless, optimal results were obtained with DNA extracted from fresh or properly stored material.

The host range of *Dothistroma* spp. includes 109 taxa within *Pinus*, *Abies*, *Cedrus*, *Larix*, *Picea* and *Pseudotsuga*. Using molecular methods, *D. septosporum* has been confirmed to infect 42 *Pinus* spp., 11 of which have been shown to also be hosts for *D. pini*. Within the 109 identified host taxa, susceptibility to *D. septosporum* ranges from slightly susceptible to highly susceptible [1]. In addition, it has been shown that on *P. radiata*, *D. septosporum* has a hemi-biotrophic life style and is able to infect and colonize host tissue asymptomatically during a biotrophic phase before switching to a necrotrophic phase [17]. If signs and symptoms of *D. septosporum* are identified in a highly susceptible species and in an area where there is an established history of disease, it may not be necessary to confirm *D. septosporum* infection using a genetic assay. However, in slightly susceptible species where morphological identification is difficult, or during a hemi-biotrophic phase prior to

symptom development, or in geographic areas without a history of the disease, the LAMP assay can rapidly provide confirmation of infection. Incorporating a LAMP diagnostic into surveillance could improve disease control as management activities and could be initiated before the pathogen has spread widely.

An advantage of the LAMP approach is that the amplification temperature is constant, making the technology suitable for point-of-care applications. Portable equipment, such as the Genie® III, allows these DNA-based diagnostics to be performed outside of a laboratory setting. Compatible with a point-of-care diagnostic, our assay returns a positive reaction in 30 to 60 min. We are currently optimizing methods for sample preparation in the field to enable tests to be conducted on site.

5. Conclusions

This LAMP assay provides an additional diagnostic assay for the detection of *D. septosporum*. Advantages of this assay include, speed, sensitivity and the potential for field deployment. This pathogen is a research and management focus for many countries [1] and as the number of diagnostic tools increases, it is hoped that management efficacy will also increase, leading to a decline in the effects of this pathogen in stands where pathogen management is necessary to achieve stand management objectives.

Supplementary Materials: The following are available online at https://www.mdpi.com/1999-4907/12/3/362/s1, Table S1. Collection locations and LAMP results for DNA extracted from cultures. Table S2. LAMP results obtained from DNA extracted from needles.

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