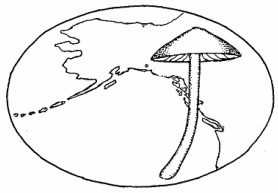
Pacific Northwest Fungi



Volume 2, Number 7, Pages 1-9
Published September 21, 2007

Septoria musiva isolated from cankered stems in hybrid poplar stool beds, Fraser Valley, British Columbia

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Callan, B.E., I. Leal, B. Foord, J.J. Dennis, and C. van Oosten. 2007. *Septoria musiva* isolated from cankered stems in hybrid poplar stool beds, Fraser Valley, British Columbia. *Pacific Northwest Fungi* 2(7): 1-9.
doi: 10.2509/pnwf.2007.002.007

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Accepted for publication September 17, 2007.

Abstract: *Septoria musiva*, a pathogen causing leaf spots and stem cankers, was isolated from cankered hybrid poplar stems (TXM 271–287 and TXM 271–286) in commercial stool beds in the Fraser Valley, southwest British Columbia (BC) in November, 2006 and January, 2007. The identity of this pathogen was confirmed both morphologically in culture and by using species-specific PCR primers, unlike earlier questionable reports of this pathogen in BC. A survey of stems larger than 1 cm diameter in one stool bed revealed that 199 out of 458 (43.4%) stems bore one or more cankers. *Septoria musiva* was isolated in culture from 44% of 50 cankered stems sampled in this stool bed. In the past, leaf spots on BC poplars have occasionally been attributed to *S. musiva* based on conidial morphology, but *S. populicola* is far more prevalent in natural and commercial cottonwood and hybrid poplar stands, and the latter pathogen is not associated with stem cankers. The conidial size range of these two species overlaps, which adds uncertainty to earlier diagnoses of *S. musiva* based on morphology alone, especially since it had not previously been isolated from cankers collected in BC. This isolation and PCR-confirmed identification of *S. musiva* documents the first record of its association with a high incidence of stem cankers in commercially grown hybrid poplars in BC.

Key words: *Septoria musiva, S. populicola, Mycosphaerella populorum, M. populicola, Davidiella populorum, Populus trichocarpa x Populus maximowiczii*, hybrid poplar, cankers, stool beds, British Columbia, PCR, culture.

Introduction: *Septoria musiva* is one of the most important fungal pathogens of hybrid poplars in eastern North America and is associated with leaf spots, premature defoliation, cankers, and stem breakage (Ostry, 1987). Highly susceptible trees with leaf infections often subsequently develop stem or branch cankers where inoculum contacts wounds, lenticels, stipules or leaf bases. In young susceptible trees, stem cankers may girdle the tree in one or two years (Sinclair and Lyon, 2005). *Septoria populicola* is known to occur at high levels in susceptible hybrid poplars in Fraser Valley commercial plantations and on nearby native *P. trichocarpa*. It causes leaf spots and defoliation during springs and early summers with higher than average rainfall. *Septoria populicola* has not been associated with cankers on any poplar hosts in BC, but may cause cankers in the field and under greenhouse conditions elsewhere (Newcombe and Ostry, 2001).

In November 2006, numerous cankers were observed on two-year-old stems in a commercial hybrid poplar stool bed in the Fraser Valley. The affected clone was *Populus trichocarpa* Torr. & Gray x *P. maximowiczii* Henry (TXM 271–287). Cankers occurred most frequently within 50 cm from ground level, and were slightly flattened, with swollen sides and sunken centers (Figs. 1, 2). The remains of small dark pycnidia were occasionally detected on the cankered bark, but no sporulation was observed on freshly collected material. Cambium tissue excised from the necrotic edge of the cankers yielded mostly *Cytospora chrysosperma* (Pers.:Fr.) Fr. and *Cladosporium* sp. on 2% malt agar, but one isolate from a single canker produced typical *Septoria* pycnidia oozing pale pinkish conidial tendrils. The conidia, which were cylindrical, hyaline and 1–4 septate, were considerably shorter on average than those produced by *Septoria populicola* Peck which commonly occurs in the Pacific Northwest (Newcombe et al., 1995), but fit within the size range for *S. musiva* Peck (Thompson, 1941). Culture morphology and canker symptoms were also consistent with published descriptions of *S. musiva* (Thompson, 1941). Poplar leaf spots and cankers collected during routine pest surveys over the past several decades at various provincial locations, (including this plantation), have been occasionally attributed to *S. musiva*. However, these diagnoses were based on solely on conidial measurements from leaf spots, and were not confirmed by culturing from cankers or other methods. Conidial size ranges for *S. musiva* and *S. populicola* overlap somewhat, adding a degree of uncertainty to diagnoses based on morphology alone (Feau et al, 2005). No widespread outbreak of cankers in BC poplars had been attributed to any *Septoria* species prior to this time.

Feau et al (2005) recently developed highly specific ITS (Internal Transcribed Spacer) primers for *S. populicola* and *S. musiva* to facilitate the accurate determination of geographic distribution and host ranges of these two species. We employed these primers as diagnostic tools, using DNA isolated from cultures to confirm the identity of the fungus isolated from two cankered full-sibling hybrid poplar clones (TXM 271–287 and 271–286) in the Fraser Valley stool bed. We also compared the *Septoria* isolates from the Fraser Valley cankers to a fresh isolate of *S. populicola* from *P. trichocarpa* leaves collected on the grounds of the Pacific Forestry Centre.

Materials and Methods: Microscopic observations were made with a Nikon Optiphot II compound microscope equipped with differential interference contrast optics. Conidia were measured at 400 X while mounted in lactoglycerol, but photographed while mounted in distilled water. Photomicrographs and field photographs were taken with a Nikon Coolpix 4500 digital camera. The first culture of *S. musiva* from TXM was isolated on 2% Difco Malt Agar, while subsequent isolations from stem cankers were made on either V–8 juice agar, or *Septoria musiva* medium (SMM, Stanosz and Stanosz, 2002). Fresh foliar samples from a *P. trichocarpa* tree on the grounds of the Pacific Forestry Centre were collected and conidial tendrils from pycnidia in leaf spots were initially streaked on to SMM, and then transferred to V–8 juice agar once colonies developed. Fifty conidia from the isolates in culture and from the fresh leaf collection were measured to obtain conidial size ranges. Leaf spots, cankers and/or dried cultures have been deposited in the Pacific Forestry Centre's Forest Pathology Herbarium (DAVFP). Accessions previously identified as *S. musiva* in DAVFP were also examined in order to compare conidial dimensions. Due to the paucity of leaf spots in the older herbarium material, only twenty conidia per collection were measured. Three DAVFP collections that were incorrectly determined were annotated and excluded.

On January 29, 2007, a trip was made to the Fraser Valley hybrid poplar nursery to collect additional samples and to conduct an incidence survey. Prior to this trip, the stool bed containing the TXM 271-287 clone was cut down. However an adjacent remaining stool bed contained the full sibling (TXM 271–286) of the cut clone, and also exhibited widespread cankering. We surveyed this stool bed (planted in 2002) by walking down each interior row of the stool bed, counting the total number of shoots (>1 cm diameter at the base) coming from the individual plants as well as recording the number of cankered stems out of this total. Outside rows were excluded, to avoid stems that could have been mechanically wounded by passing machinery.

Fifty cankered cuttings from clone TXM 271–286 were collected and brought back to the laboratory for culturing. This second stool bed was destroyed soon after, so no foliar samples could be subsequently collected. One canker from each cutting was dissected using a sterile scalpel to excise the surface bark from the distal, medial and proximal margin of the canker. The scalpel was re-sterilized and a small amount of tissue removed from a flap cut under each exposed necrotic edge of the cambium. The three pieces of excised tissue were placed on a single Petri plate containing SMM and incubated at 20° C under fluorescent light supplemented by daylight from a nearby window.

DNA was extracted from the original isolate from clone TXM 271-287 (DAVFP 29313), three isolates randomly chosen from the cultures obtained from clone TXM 271–286 (DAVFP 29316, 29317 and 29318), and the *S. populicola* isolate from the PFC grounds (DAVFP 29315). Mycelium and conidia of these isolates were removed from agar plates and ground in liquid nitrogen using a mortar and pestle. DNA was extracted using a Qiagen DNeasy plant mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's directions. The DNA concentration was determined using a NanoDrop ND–1000 spectrophotometer prior to performing PCR analysis.

The ITS ribosomal region from each of the *Septoria* isolates was amplified with the following species–specific primer sets developed by Feau et al. (2005): Smusf and Smusr for *S. musiva*; Spopf and Spopr for *S. populicola*; Spnf, and Spnr for *S. populi* Desm. (occurrence of the latter species in North America is uncertain). PCR amplification was performed in 25 µl reactions containing 12.5 µl of Qiagen's Taq Master Mix, 40 µg bovine serum albumin, 1 µM of each appropriate primer

and 5 µl (1ng/µ of DNA template. PCR cycles were carried out according to the modifications that were made at the University of Wisconsin-Madison as described in Feau et al. (2005) in a Biometra T–gradient Thermocycler. The PCR products were separated by electrophoresis on a 1% agarose gel in 1x TAE buffer containing 0.25 µg/ml ethidium bromide. The gel, which ran at 80 V for 1 hr, was photographed under UV light.

Results: Conidial size ranges from the *Septoria* cultures obtained from the original cankered poplar tissue on clone TXM 271–287 collected in the Fraser Valley (DAVFP 29313) were (22–) 28–58 (–62) x 3.5–4 µm (Fig. 3), with the average spore length being 40.28 µm, just slightly longer on average than described by Thompson (1941) and much shorter than conidia of *S. populicola* (Thompson, 1941). The *S. populicola* isolate collected from *P. trichocarpa* on the PFC grounds (DAVFP 29315) produced conidia that were morphologically typical for this species (Fig. 4, Table 1). However, herbarium specimens collected earlier in BC and identified as *S. musiva* yielded conidia with size ranges and mean lengths that were intermediate between those of *S. musiva* and *S. populicola* (Table 1).

A total of 458 stems of clone TXM 271–286 were examined in the surveyed stool bed. Of these 198 (43.4%) bore one or more cankers. Out of the fifty cankers sampled, 22 (44%) yielded cultures which were morphologically identified as *S. musiva*, with similar conidial size ranges to those described above from the original isolate.

The original isolate from clone TXM 271-287 and three isolates randomly chosen from the cultures obtained from clone TXM 271–286 were all confirmed to be *S. musiva* using PCR amplification of the ITS region. Figure 5 (Lanes 1-4) shows that the DNA from all *S. musiva* isolates was amplified exclusively with the *S. musiva*-specific primers (Smus f/r), and was not amplified with the sets of primers for the other two *Septoria* species. The DNA from the *S. populicola* isolate (Lanes 5, 12, 19) was amplified exclusively with the *S. populicola*-specific primers (Spop f/r). No DNA from any of the isolates was amplified with the *S. populi*-specific primers (Lanes 15-20). The 319 bp fragment amplified from the DNA of the original *S. musiva* collection (DAVFP 29313 on TXM 271-287) was sequenced and registered in the National Center for Biotechnology Information (NCBI) database as EF612702. The results of a BLAST search of the NCBI database using this base sequence resulted in a 99% match with 0% gaps corresponding to the following accession numbers (registered as *M. populorum*): DQ029124.1, DQ029123.1, AY752867.1, AY752864.1, AY752865.1, AY555277.1, AY549467.1, AY549466.1, AY549465.1, and AY549464.1.

Material examined: *Septoria musiva* Peck (deposited as *Mycosphaerella populorum* G.E. Thompson): **Canada**. British Columbia: DAVFP 23833; DAVFP 24241; DAVFP 24242; DAVFP 24197 [Note - no pycnidia or conidia were found on this specimen when it was examined during this study]; DAVFP 24201; DAVFP 24202; DAVFP 29313; DAVFP 29316; DAVFP 29317; DAVFP 29318.

Septoria populicola G.E. Thompson (deposited *as Mycosphaerella populicola* G.E. Thompson): **Canada**. British Columbia: DAVFP 29315.

Specimens originally identified as *S. musiva* and redetermined as *S. populicola* or other species: DAVFP 23873; DAVFP 24128; DAVFP 24277.

Detailed georeferenced specimen label data are available on-line at: http://www.pfc.cfs.nrcan.gc.ca/biodiversity/herbarium/voucher_specimens_e.html

Discussion: The majority of *Septoria* leaf spots collected in BC during past pest surveys have been attributed to *S. populicola*. Occasionally, leaf spot collections bore *Septoria* conidia with

shorter than average lengths for *S. populicola* and were consequently identified as *S. musiva*. Microscopic examination of these older herbarium specimens deposited in DAVFP revealed that conidial size ranges and average lengths were actually often intermediate between the two species, thus raising some uncertainty regarding the earlier morphological diagnoses of *S. musiva* in BC. None of the field observations linked to these earlier foliar collections indicated that cankers were also present on the affected trees. A study of fresh *Septoria* leaf spots collected from *P. trichocarpa* at 61 sites throughout the Pacific Northwest, confirmed the presence of *S. populicola* only, based on conidial mean length measurements ranging from 54–80 µm (Newcombe et al., 1995). Stem cankers have never been associated with infection by *S. populicola* in BC. We believe that our documentation of extensive stem cankering in these Fraser Valley TXM hybrid poplars, and the frequent isolation of the pathogen, whose identity was confirmed both by conidial morphology and PCR, represents the first reliable record of stem canker caused by *S. musiva* in BC.

We have not observed the presence of the teleomorph of *S. musiva* (*Mycosphaerella populorum* G.E. Thompson) in the affected stool bed. In BC, overwintered hybrid poplar and cottonwood leaves frequently bear pseudothecia of *Davidiella tassiana* (De Not.) Crous & U. Braun, the sexual state of *Cladosporium herbarum* (Pers.:Fr.) Link (Callan, 1998). Published descriptions of *M. populorum* (Thompson, 1941) and *D. tassiana* (Corlett, 1991) indicate that the ascospore sizes of the two species are very similar, with ascospores of the former measuring 16–28 x 4.5–6 µm and the latter measuring 16–29 x 4.5–8 µm. A recent monograph of *Mycosphaerella* by Aptroot (2006) resulted in the confusing redisposition of both *M. populicola* G.E. Thompson and *M. populorum* to the genus *Davidiella* Crous & U. Braun. *Davidiella* is separated from *Mycosphaerella*, from which it is otherwise morphologically identical, by the fact that *Davidiella* has *Cladosporium* anamorphs (Braun et al., 2003). Further observation and culturing of pseudothecia from overwintered leaves might confirm the presence of the teleomorphs of *Septoria* species on BC poplars, and determine the level of their occurrence in native stands and hybrid plantations. Future surveys should also be undertaken to determine if the pathogen has spread to adjacent hybrid poplars in this nursery or nearby native *P. trichocarpa* stands. Although pure *P. deltoides* trees in their natural range are resistant to stem cankers caused by *S. musiva*, *P. trichocarpa* and many of its hybrids with other poplar species, particularly *P. maximowiczii*, are susceptible when planted in north central and eastern North America (Hansen et al, 1994; Newcombe and Ostry, 2001). In BC, an estimated 80% of the 12,000 hectares planted to hybrid poplar as of 2004 have a *P. trichocarpa* parent (Cees Van Oosten, pers. comm.). Growth conditions in poplar stool beds and biofuel production plantations (close spacing of many young stems; coppicing) are highly favorable for development of cankers in areas where the pathogen is established (Ostry, 1987). Surface contamination of cuttings by conidia is possible in stool beds with high levels of inoculum, resulting in asymptomatic, but contaminated propagative material. Incipient infections of *S. musiva* are also easily missed during the processing of propagative stock (Ostry, 1987). Choice of canker-resistant hybrid clones and prevention of further spread of the pathogen via restricted movement of cuttings from the affected area should now be considered by regional nursery and plantation managers.

Acknowledgements: The authors would like to thank Dan Carson and Gashaw Abebe for their assistance during specimen collection and field surveys. We also thank Phyllis Dale and Heather Wurtele for their helpful critiques of the manuscript.

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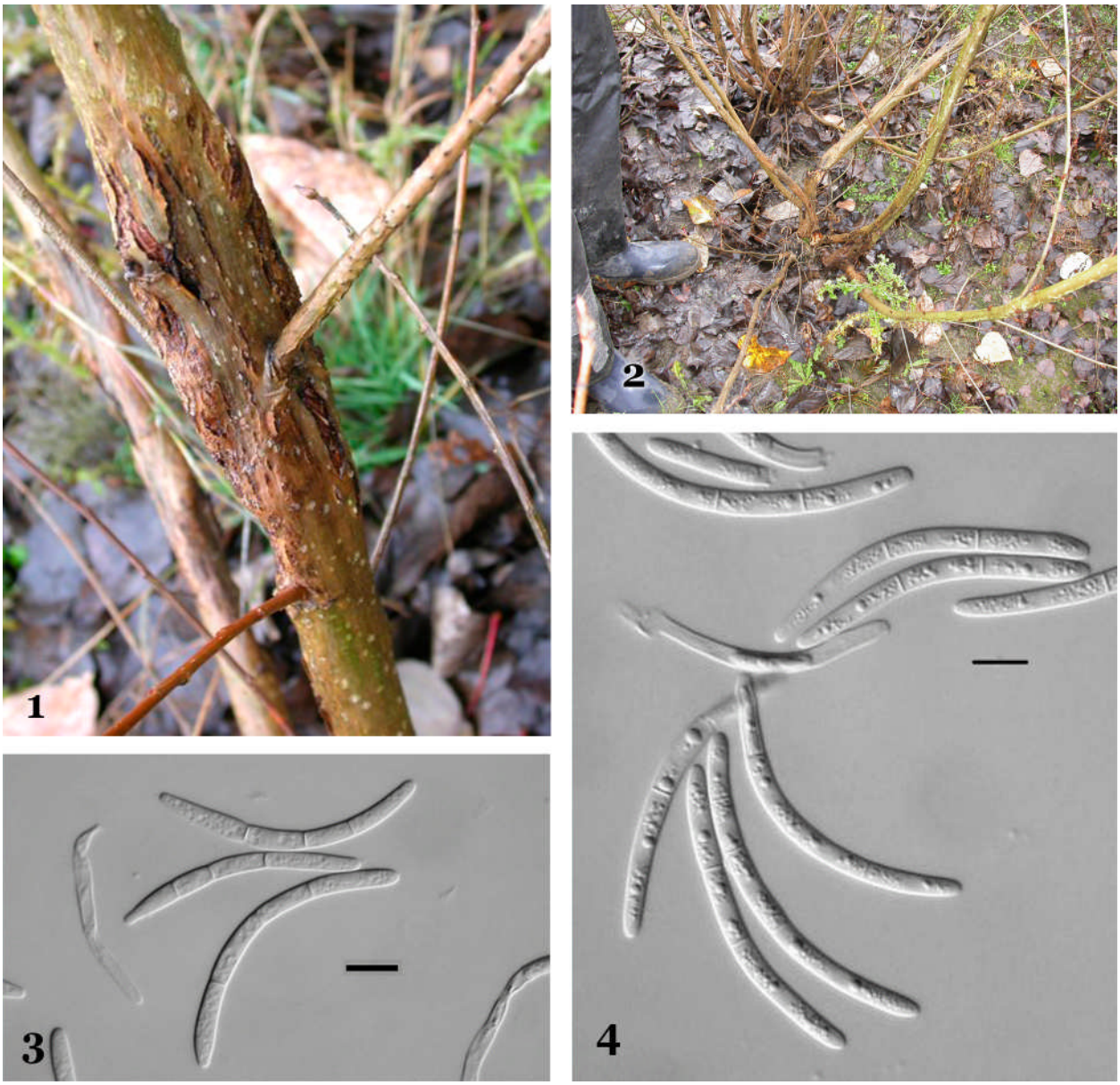
Source	**Conidial size range (l x w; µm)**	**Mean conidial size (l x w; µm)**	**Symptom**
S. musiva DAVFP 23833 (Broman Lake, BC, 1988)	(40–)45–66(–72) x 3(–4)	52.00 x 3.20	Leaf spot
S. musiva DAVFP 24201 (Fraser Valley, BC, 1991)	(38–)40–60(–63) x 4	51.05 x 4	Leaf spot
S. musiva DAVFP 24202 (Fraser Valley, BC, 1991)	(39–)46–64(–66) x 4	54.45 x 4	Leaf spot
S. musiva DAVFP 24241 (Burns Lake, BC, 1991)	(40–)46–58(–60) x 4	51.15 x 4	Leaf spot
S. musiva DAVFP 24242 (Rosswood, BC, 1991)	(43–)52–64(–65) x (3.5–)4(–4.5)	54.50 x 4	Leaf spot
S. musiva DAVFP 29313[1] (Fraser Valley, BC, 2006)	(22–) 28–58 (–62) x 3.5–4	40.28 x 3.89	Canker
S. musiva (Thompson, 1941)	28–54 x 4	37 (length only)	Leaf spot/ Canker
S. populicola DAVFP 29315 (PFC Property, 2007)	(50–)52–72(–78) x 4	60.63 x 4	Leaf spot
S. populicola (Callan, 1998)	60–110 x 3.5–4.5	-	Leaf spot
S. populicola (Newcombe et al., 1995)	54–80 (length only)	-	Leaf spot
S. populicola (Thompson, 1941)	45–80 x 3.5–4.5	62 (length only)	Leaf spot

Table 1. Comparison of conidial dimensions from Fraser Valley collections, earlier herbarium specimens, and published records. [1]Conidia obtained from culture. All other conidia obtained from leaves.



Figs. 1-4. Field symptoms of *S. musiva* and conidial morphology of *S. musiva* and *S. populicola*. Figs. 1, 2. Canker and stem breakage symptoms caused by *S. musiva* on stems of TXM 271-287 in a Fraser Valley stool bed, November 14, 2006. Fig. 3. Conidia from *S. musiva* culture DAVFP 29313. Fig. 4. Conidia from *P. trichocarpa* leaf spots caused by *S. populicola* (DAVFP 29315). Bars = 8 µm.

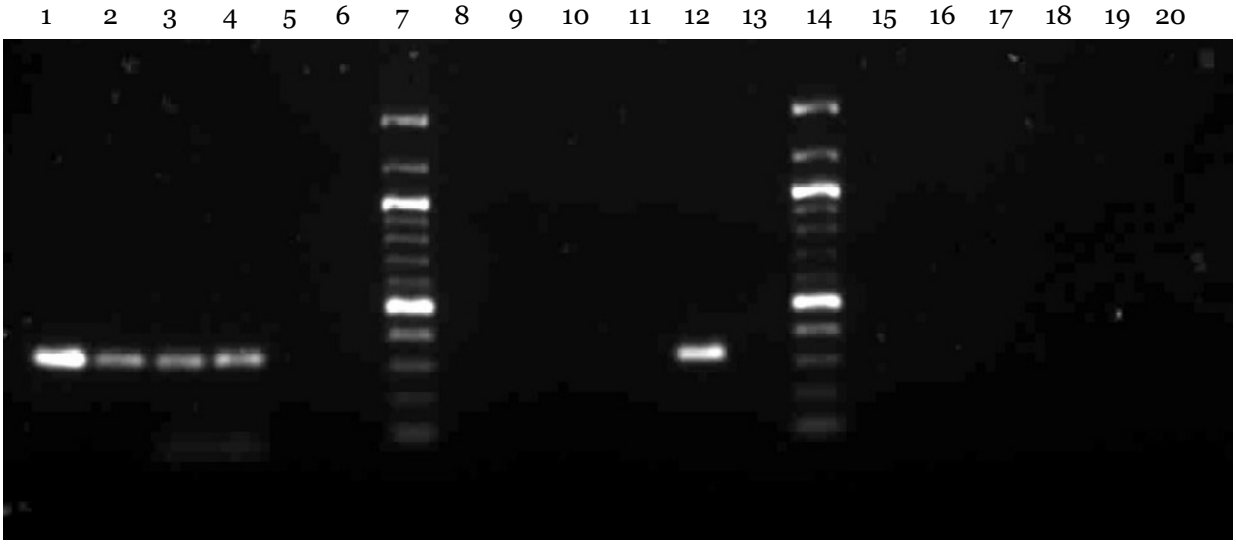


Fig. 5. PCR amplification products of the ITS region of *S. musiva* and *S. populicola* isolates showing the 319 bp fragment generated. Lanes 1, 8, 15 – *S. musiva* DAVFP 29313; Lanes 2, 9, 16 – *S. musiva* DAVFP 29318; Lanes 3, 10, 17 – *S. musiva* DAVFP 29317; Lanes 4, 11, 18 – *S. musiva* DAVFP 29316; Lanes 5, 12, 19 – *S. populicola* DAVFP 29315; Lanes 6, 13, 20 – Control Blank; Lanes 7, 14 – 100 bp Ladder (New England Biolabs). Primer Sets: Lanes 1-6 – Smus f/r; Lanes 8-13 – Spop f/r; Lanes 15-20 – Spn f/r.