Fungal Spores in Insect Trapping Fluids: Simultaneous Sampling for Insects and Pathogens

Jean A Berube (jean.berube@canada.ca)
Canadian Forest Service https://orcid.org/0000-0001-8941-0943
Jeremy D. Allison
Canadian Forest Service
Kate Van Rooyen
Canadian Forest Service
Cory Hughes
Canadian Forest Service
Patrick N. Gagné
Canadian Forest Service
Jon Sweeney
Canadian Forest Service

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Abstract

Surveillance for early detection of non-native, invasive pathogens requires simple, sturdy, and easy to use collecting devices. In this study, we compared the fungal species detected in wet collection cups of Lindgren traps versus those detected on slides with oiled cheesecloth as aerial spore collectors. DNA was extracted and amplified from both using the primers ITS1F -ITS7G, and Illumina sequencing was used for metabarcoding of fungi present in samples. In 90 samples there were 1277 fungal OTUs. For fungal OTUs only detected by one collection method, insect traps had three times the number of fungal OTUs compared to slides, and this pattern persisted when analyses were restricted to pathogens and forest pathogens. Annually, thousands of insect traps are deployed in North America and the associated trap fluids have added value in forest disease research and monitoring.

Key Message

- Of 1277 fungal OTUs collected in this study, 476 were identified from insect trap fluid but not aerial spore collectors. In contrast, 132 OTUs were identified in spore collectors but not insect trap fluids.
- Screening insect trap fluids for fungal spores may represent an opportunity for significant cost and labour savings in operational forest biosecurity programs.

Introduction

Forest ecosystems provide a diverse number of goods (e.g., food, timber, fuel) and services (e.g., biodiversity maintenance, carbon storage, nutrient cycling, water and air purification) (Vira et al. 2015; Wingfield et al. 2015). With growth in human populations, the significance of these goods and services, as well as disturbances that threaten the health of forest ecosystems, are expected to increase. Invasive insects and pathogens are one of the most significant threats to the health of forest ecosystems globally (Roy et al. 2014) and the movement of species outside of their native range is expected to continue to increase (Seebens et al. 2017).

Although the impacts of biological invasions are realized regionally, at least initially, their causes are primarily international (i.e., trade and transport pathways) and unintended (Westphal et al. 2008; Hulme 2009). As a result, the emphasis of many national forest biosecurity programs is prevention (Allison et al. 2021). A large network of international and regional regulatory instruments have been developed to try to restrict the movement of species outside of their native ranges (Moore 2005; Ormsby and Brenton-Rule 2017). These regulatory instruments attempt to manage biosecurity threats in the area of origin as well as in the invaded range (Ormsby and Brenton-Rule 2017).

There is some evidence that biosecurity efforts have been successful. For example, Haack et al. (2014) estimate that from 2003 to 2009, ISPM 15 contributed to a 36-52% reduction in infestation rates of inspected wood packaging coming into the United States. Despite these efforts and their associated reductions in propagule pressure, levels of globalization and trade continue to increase and as a result species continue to arrive and establish outside of their native ranges (Chapman et al. 2017; Seebens et al. 2017). For invasive species with significant ecological and economic impacts, eradication from the invaded range often provides significant economic return on management efforts (Sharov and Liebhold 1998; Myers et al. 2000; Liebhold and Tobin 2008). Although eradication can be difficult to achieve, several examples of successful eradication programs exist, including forest pests (e.g., Asian longhorned beetle (CFIA 2020; Allison et al. 2021)).

One of the primary determinants of the success of eradication efforts is the size of the area infested and associated population densities, both of which often increase with the time since establishment (Myers et al. 2002; Brockerhoff et al. 2010; Liebhold et al. 2016). The availability of sensitive, low-cost surveillance tools is critical for assessing population densities, delimitation of the exotic in the invaded range and assessing the efficacy of management interventions. The success of containment and long-term control programs that attempt to limit spread and population densities within the invaded range also depends on the availability of surveillance tools.

Although economic analyses indicate that the benefits of surveillance programs exceed their costs (Epanchin-Niell et al. 2014), surveillance programs are costly (Blackburn et al. 2016). In part, the large investment required for effective surveillance is driven by the fact that optimal sensitivity requires use of multiple surveillance tactics (Yemshanov et al. 2014). This is because surveillance programs are often designed to target communities of species (the identity of which are often unknown *a priori*) and the performance of surveillance tools varies among taxa (e.g., Allison et al. 2014; Dodds et al. 2015; Allison and Redak 2017). Several studies have demonstrated that intercept traps can be co-baited with lures that target different insect communities without significant reductions in efficacy for target insect taxa (e.g., Witzgall et al. 2010; Rassati et al. 2014; Chase et al. 2018; Marchioro et al. 2020). This pyramiding of attractants, facilitates the use of one trap to simultaneously sample multiple species, significantly reducing labour and material costs.

Plant diseases are increasing in frequency in forest ecosystems (Desprez-Loustau et al. 2007; Santini et al. 2013). Many of these diseases are caused by exotic pathogenic fungi and oomycetes and have significant ecological and economic impacts in the invaded range (Desprez-Loustau et al. 2007; Wingfield et al. 2015; Ramsfield et al. 2016). Surveillance of plant pathogens and the diseases they cause is critical to early detection of invasive species and changes in population densities of naturalized and native pest species (Parnell et al. 2017). As with insect pests, once plant pathogens are established across a broad range and/or begin to experience rapid population growth, the likelihood of successful management is low. The importance of surveillance for forest pathogens is further increased by the fact that few control tactics are available to manage pathogens in forest ecosystems. Surveillance programs in forest ecosystems typically target pathogens or insects. This is primarily because the collectors for fungal spores cannot capture and retain insects; however, intercept traps used to survey insects can collect spores either through random alightment of spores into the trap collection reservoir or via the body of captured insects. The aim of this study was to compare the efficacy of passive spore collecting devices and insect intercept traps with wet

collection cups for sampling fungal species. Secondarily, we tested the effect of site type, rural versus urban, on abundance and number of fungal species detected.

Materials And Methods

Insect traps and slides were deployed in wooded areas at one urban and one rural site located <15 km apart in each of three Canadian cities: Sault Ste. Marie, Ontario; Fredericton, New Brunswick; and Halifax, Nova Scotia. Within each city, urban sites were located relatively close (< 2 km) to industrial parks whereas rural sites were located 6–15 km distant from these areas. At each of the six sites, we set up two insect traps and two slide collectors in a paired design with traps and slides < 5m apart within pairs and replicate pairs separated by about 30 m. Preservative fluids and slides were collected weekly or biweekly (3–4 times) from 1–28 August 2018 in New Brunswick and Nova Scotia, and weekly from 16 August–13 September 2018 in Ontario. The samples were shipped with icepacks and kept frozen at −20°C until extraction. A total of 46 slides and 44 trap preservative fluids were collected.

Black Lindgren 12-funnel traps (Synergy Semiochemical Corp., Burnaby, BC, Canada) were suspended from rope tied between two trees such that the trap was at least 1m from the nearest tree and the collection cup 30–50 cm above the ground. Traps were treated with 50% Fluon diluted in water to increase efficiency of capturing wood boring beetles (Allison et al., 2016). The collecting cups contained commercial RV antifreeze as preservative fluid (WinterProof Water System Antifreeze, 10–30% ethanol, 1–5% propylene glycol, Recochem Inc., Montreal, QC, Canada) with a drop or two of liquid dish detergent to reduce surface tension in the NB and NS sites. Each funnel trap was baited with four semiochemical lures known to increase captures of several species of bark and wood boring beetles in the families Cerambycidae and Curculionidae (subfamily Scolytinae), i.e., monochamol, ipsenol, alpha pinene and ethanol (Allison et al. 2003; Miller et al. 2016, Boone et al. 2019; Flaherty et al. 2019).

A new model of aerial spore collector was developed using technology from the 1960's for its simplicity to fabricate and use by non-scientists. It consists of Grade 50 double layer cheesecloth (Uline, Pleasant Prairie, WI, USA) impregnated with silicone oil #378399 (Sigma) at 60 g per m^2 mounted in 35 mm projector slides. The slides are held in place by an alligator clip inside a 25.4 cm diam. airport wind indicator (Airport Windsock Corporation, Lake City, MN, USA). The slide was positioned 2 m above ground.

Preservative fluids from each trap collection (approximatively 250 ml) from Ontario were filtered on 25 mm diam, 2.7 µm pore size Glass Microfiber GF-D (Whatman, Buckinghamshire, UK) using vacuum, whereas Nova Scotia and New Brunswick fluids were filtered on 90 mm diam., Whatman #1 qualitative filter papers 11 µm pore size (Whatman, Maidstone, UK). Filters and cheesecloth were sampled using a 6 mm paper punch. One 6 mm disk per filter and cheesecloth slide was ground using a Christison M3 Mixermill with a tungsten bead, twice for 2 min at 30 hertz with extraction buffer. Extraction was done with the Plant DNeasy mini kit (Qiagen Inc., Valencia, CA, USA) according to manufacturer instructions. One microliter of the eluate was used as genomic DNA (gDNA) template for Polymerase Chain Reaction (PCR).

In order to obtain a comprehensive data set of all fungal DNA present in our samples, a High Throughput Sequencing (HTS) method based on the Illumina Miseq sequencing system was used. DNA amplification, primer constructs, purification and sequencing was done as described in Bérubé et al. (2018). The primers ITS1F and ITS7G were used to amplify the ITS regions of the ribosomal DNA fragment (ITS1- 5.8S) for metabarcoding of fungi present on filters and slides.

Each of the 90 samples was tagged with differing indices, PCR amplified separately and then tagged amplicons were pooled in equimolar amounts of 4 ng DNA per sample. Final quantification, primer dimer removal and amplicon quality check were done with an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Pooled DNA samples were sent to the Next- Generation Sequencing Platform, Genomics Centre, CHU de Québec- Université Laval Research Centre, Quebec City, QC, Canada, which performed paired-end 300 bp sequencing using MiSeq Reagent Kit v3 (600- cycles) through an Illumina MiSeq system.

A bioinformatics treatment of HTS DNA sequences was executed to create Operational Taxonomic Units (OTUs), our proxies for fungal species (Huse et al. 2010; Kunin et al. 2010). Sequence analysis was done as described in Bérubé et al. (2018). In order to minimize the loss of rare and targeted fungal species, standard bioinformatic tools like QIIME2 and DADA2 were not used. Instead a custom bioinformatic pipeline that minimizes losses of singletons and rare OTUs was used (Gagné et al. 2020). Sequence sets were organized into clusters with USEARCH 64 bit v8.0.1623 with a sequence similarity threshold of 97% to agglomerate reads and form the OTUs, the most abundant sequence types serving as cluster seeds. As no single similarity threshold will accurately reflect the species level throughout the fungal kingdom, a 3% dissimilarity cut-off was selected as a compromise in order to avoid overestimating fungal diversity versus masking rare OTUs and putative new emerging fungal pathogens (Nilsson et al. 2008; Schoch et al. 2012; Huse et al. 2010). Representative sequences, which are the most frequent sequence in each OTU, were extracted and then screened against Genbank nr/nt database using BLAST to identify rare, and potentially new emerging invasive fungal species not found in curated databases. Output Excel files were then organized alphabetically by Latin names and parsed for plant pathogens of interest and those on the quarantine species list of Canada and other industrialized countries.

Cross-talk is a phenomenon that occurs when a DNA read is assigned to an incorrect sample (Edgar 2018). When dealing with experiments attempting to identify emerging pests, incorrect assignment of DNA reads can lead to troublesome conclusions about its presence and distribution. To alleviate this problem, positive controls of targeted pests were not used in this trial in order to avoid cross-talk contaminations. One unused index was also sent as sequencing blanks during the sequencing output processing to quantify cross-talk.

Statistical Analysis

The primary output Excel file was compressed according to Genbank accession number to fuse redundant OTUs created by our bioinformatic method. OTUs that were ≥ 97% similar were pooled. Data for OTUs with less than 10 reads in a weekly sample were converted to zeros. We then generated three groupings of OTUs for analysis: 1) forest pathogens, 2) plant pathogens (which included forest pathogens as well as non-forest plant pathogens); and 3) all fungal OTUs (including plant pathogens). DNA read counts in the weekly samples were summed over the entire 4-week sampling period to yield a balanced data set with one cumulative count of each OTU for each collection method, site type, site and replicate.

Because the pore diameter of filter papers used to filter trap fluids was larger in New Brunswick and Nova Scotia (11µm) than in Ontario (2.7µm), some fungal species (i.e., with spores < 11 µm diameter) present in trap fluids in New Brunswick and Nova Scotia may have been missed. If that were true we would expect the relative performance of trap fluids vs. aerial spore collectors to be greater in Ontario vs. the other two sites. To test this, we ran separate 2 x 2 contingency table analyses on the proportions of fungal species detected by trap fluids vs. aerial spore collectors at each of the three sites, as well as on data pooled from all sites, and then tested for differences in performance among sites using the Chi square heterogeneity test (Zar 1999, pp. 500–502). We ran separate analyses on each of our three groups of fungi, i.e., all fungi, plant pathogens, and forest pathogens.

We then ran generalized linear mixed-effect models (PROC GLIMMIX) in SAS 9.4 for Windows (v. 6.2.9200, ©2002–2012, SAS Institute Inc., Cary, NC, USA) to test the effects of collection method (aerial spore collectors vs. insect trapping solution), site type (forest vs. urban), collection method x site type interaction, and site (Sault Ste. Marie, Fredericton, Halifax) on species richness and abundance of forest pathogens, plant pathogens, and all fungal species, and abundance of the more common individual forest pathogens. Collection method and site type were considered fixed effects and sites were considered random effects. We ran the models with Gaussian (on both raw and log (y+1) transformed data), Poisson, and negative binomial distributions and report results from the model with best fit (negative binomial in all cases) as determined by lowest value of the corrected Akaike Information Criterion (AICc). Least-square means were compared using the Tukey-Kramer method with experiment-wise error controlled at α = 0.05, but means and standard errors are reported on raw (species richness) or log(y+1) transformed data (abundance). Of the total of 101 forest pathogens, only 25 of the most common were analyzed by generalized linear mixed models to test for effects of collection method and site type on their individual abundance; high numbers of zero counts in the remaining OTUs made them inappropriate for analysis by generalized linear models.

Results

We detected a total of 1277 fungal OTUs, 220 of which were plant pathogens, and 101 of these were forest pathogens (Table 1). Trap fluids detected 90% of all fungi collected by both methods including 476 OTUs that were not detected by aerial spore collectors (Table 1; Fig. 1A). Aerial spore collectors detected 63% of total fungi collected, including 132 OTUs not detected in trap fluids; 669 OTUs were detected by both methods (Table 1, Fig. 1A). Sixty-two of 220 plant pathogens were detected exclusively by trap fluids compared to only 15 plant pathogens detected exclusively by aerial spore collectors (Fig. 1B). Of 101 forest pathogens, 27 were detected exclusively by trap fluids and 6 exclusively by aerial spore collectors (Fig. 1C).

At each of the three sites, a significantly greater proportion of fungi were detected by trap fluids (77–90%) than by aerial spore collectors (46–61%) ($P < 0.0001$, Fisher Exact test) (Table 1). Trap fluids also outperformed aerial spore collectors in proportions of plant pathogens and forest pathogens detected in Nova Scotia and Ontario ($P < 0.005$) but not in New Brunswick ($P \geq 0.13$) (Table 1). There was significant heterogeneity among sites in the proportion of species detected by trap fluids vs. aerial spore collectors for total fungi ($\chi^2 = 297$, $P < 0.0001$), plant pathogens ($\chi^2 = 59$, $P < 0.0001$), and forest pathogens ($\chi^2 = 27$, $P < 0.0001$), indicating the relative performance of collection methods varied among sites.

Species richness of fungi and pathogens

Collection method significantly affected the number of forest pathogens ($F_{1,18} = 21.9$, $P = 0.0002$), plant pathogens ($F_{1,18} = 17.3$, $P = 0.0006$) and total fungi ($F_{1,18} = 32.5$, $P < 0.0001$) detected, with trap fluids detecting 1.6–1.8 as many species as aerial spore collectors (Figure 2A–C; Supplementary Table 1). Species richness of forest pathogens, plant pathogens and total fungi were not significantly affected by site type ($F_{1,18} \leq 3.25$, $P \geq 0.09$) nor by the interaction between site type and collection method ($F_{2,18} \leq 1.51$, $P \geq 0.23$).

Abundance of fungi and pathogens

Collection method also significantly affected the abundance of forest pathogens ($F_{1,18} = 7.02$, $P < 0.016$) and total fungi ($F_{1,18} = 34.6$, $P < 0.0001$) with trap fluids outperforming aerial spore slide collectors (Figure 2D,F; Supplementary Table 1). However there was a significant interaction between collection method and site type for total fungal abundance ($F_{2,18} = 6.48$, $P = 0.02$); mean abundance of fungi in trap fluids was greater than that in aerial spore collectors in rural sites but not urban sites (Figure 1F). Site type also affected the abundance of forest pathogens ($F_{1,18} = 7.13$, $P = 0.016$) with more detected in rural than urban sites (Figure 2D). However, abundance of plant pathogens was not significantly affected by collection method ($F_{1,18} = 0.15$, P 0.70), site type ($F_{1,18} = 0.04$, $P = 0.83$) or their interaction ($F_{1,18} = 2.17$, P =0.16) (Figure 2E).

There was a significant effect of collection method on abundance of 13 of 25 species of forest pathogens analyzed ($F_{1,18} \geq 4.89$, $P \leq 0.04$) (Supplementary Table 2). Trap fluids detected more DNA than aerial spore collectors did for 11 of 13 forest pathogens whereas the reverse was true for 2 of 13 forest pathogens (Table 2). Site type significantly affected abundance of four forest pathogens ($F_{1,18} \geq 4.43$, $P \leq 0.05$) and in each case, more DNA was detected in the rural site than the urban site (Table 2).

Discussion

Two of the biggest threats to the health of forest ecosystems are invasive species (primarily pathogens and insects) and climate change (Trumbore et. al. 2015). Although the impacts of climate change on biological invasions can be difficult to predict (Ramsfield et al. 2016), it is clear that climate change has resulted in altered distributions for many taxa (Parmesan and Yohe 2003; Chen et al. 2011; Pecl et al. 2017) and can negatively affect tree resistance (Jactel et al. 2012). As a result, increased global trade and anthropogenic effects on global climate will increase the need for biosecurity programs. Surveillance of forest pests is an important component of forest biosecurity but is costly and most programs target insects and pests independently. This study has demonstrated that it is possible to simultaneously sample fungal and insect communities in forest ecosystems without a loss in program sensitivity. In fact, this study found that of the 1277 fungal OTUs detected, approximately 50% were detected in both aerial spore detectors and insect trapping fluids, 40% were detected only in trapping fluids compared to 10% that were detected only by spore collectors. Similar patterns were observed when comparisons were restricted to plant pathogen and forest plant pathogen species. The ability to use one trap to simultaneously sample for forest insects and pathogens without a loss in sensitivity represents a major programmatic advance.

Many insect surveillance programs use intercept traps with wet cups (e.g. Rabaglia et al. 2019) and the preservative fluid in these traps has potential for the surveillance of fungal spores. The idea of using preservative trap fluids for metabarcoding of invertebrates is not new (Zizka et al., 2019) and fungal species have previously been detected in insect trapping fluids (Tremblay et al. 2018). Insect trap fluids have also been used to characterize biodiversity associated with insects (e.g., bacteria, Linard et al. 2016). Similarly, other studies used crushed insect specimens to extract bacterial DNA for metabarcoding (Ravenscraft et al., 2019; Malacrino et al., 2017). Many metabarcoding studies have used classical aerial spore collecting devices such as active rotating-arm devices (Botella et al., 2019), sticky Ionic traps (Redondo et al., 2020), Hirst-type spore traps (Dananché et al., 2017; Aguayo et al., 2021), Burkard volumetric spore traps (Nicolaisen et al., 2017) or passive collecting devices such as rainwater traps (Crandall et al., 2020; Castano et al., 2019). This is the first comprehensive study to compare insect trap fluids and classical spore collecting devices.

There are at least two possible reasons why a greater number of fungal species were observed in trap fluids than the slide aerial spore collectors. The first could be the difference in trapping surface area. The funnel traps have more than 200 fold the silhouette surface area (1393 cm^2) (McIntosh et al., 2001) for intercepting wind-borne spores than the slide aerial spore collector (8.4 cm^2). Secondly, while semiochemical-baited traps actively attract many beetle species (e.g., *Monochamus* spp., *Ips* spp., *Dendroctonus* spp.; Allison et al. 2012; Flaherty et al. 2019), which carry fungal spores, including potentially damaging Ophiostomatales blue stain fungi (Jacobs et al., 2003; Jankowiak et al., 2007; Kirisits, 2007; Min et al., 2009; Yamaoka et al., 1997), aerial spore collecting is a passive process. If the higher fungal diversity in trap fluids *vs.* aerial spore collectors is due to the insects captured, species more abundant on spore collectors likely do not rely on insect vectors for dispersal. Of the two species that were more common in aerial spore collectors at least one (*Ceratocystis* sp.) comes from a genus known to be vectored by insects. While some of the eleven species that were more common in the trap fluid are known to be vectored by insects (e.g., *Ophiostoma* sp.), some are not (Table 2), suggesting that multiple mechanisms likely explain the treatment effects observed in this study.

Site type had no effect on species richness of forest pathogens, plant pathogens or total fungi and affected only the abundance of forest pathogens detected, with more detected at rural than urban sites. By contrast, Rassati et al. (2015) found more species of bark and wood boring beetles in traps that were placed in forests surrounding ports compared to traps placed directly inside ports and showed that species richness was positively related to the proportion of forest cover in the surrounding landscape. The minor effects of site type on fungal pathogens detected in our study may be due in part to the fact that both urban and rural sites were forested areas, so the proportion of forest cover (though not quantified) surrounding our traps was qualitatively similar at both site types.

The contingency table analysis indicated that the relative performance of trap solution *vs.* slide collectors for detecting species of plant pathogens was greater in Ontario than in New Brunswick and Nova Scotia. This was likely due in part to the smaller pore size of filters used to strain the trap solution in Ontario (2.7 µm) than in New Brunswick and Nova Scotia (11 µm). For example, the ellipsoidal spores of *Cladosporium cladosporioides,* which measure 3–7 µm long and 2–4 µm wide (Reponen et al., 2001), were detected with all aerial spore collector samples at all three sites and in all trap solution samples from Ontario but in only 2 of 12 and 2 of 16 trap solution samples from Nova Scotia and New Brunswick, respectively. Our results suggest that the smaller pore size filters should be used in the future to reduce the risk of missing fungal pathogens that may be present in trap solutions. Future studies should explore this question further by straining trap solution samples sequentially through filters of decreasing pore size to determine which species are missed by the coarser filters.

Cross-talk associated with High Throughput Sequencing can be troublesome, inducing nearly 1% of DNA reads to be incorrectly assigned to a sample (Edgar 2018). When dealing with a moving front of large populations of an invasive pest, cross-talk could lead to incorrect conclusions about actual distribution in the invaded range. However when determining the range of a recently introduced pest with an extremely small number of individuals, the probability that cross-talk affects that species DNA read assignment is very small, indeed less than 1% of OTUs in our studies were subjected to cross-talk.

The common association of many forest fungal pathogens with bark and wood boring beetles (Krokene and Solheim 1998; Six and Wingfield 2011) combined with the diversity of beetle species captured in semiochemical-baited traps commonly used for surveillance in biosecurity programs (Rabaglia et al. 2019) and the results of this study, suggest that insect trap fluids have excellent utility for detection of non-native forest pathogens. Annually, thousands of wet-cup traps are deployed in North America for monitoring and early detection of potentially invasive forest insect pests and trap fluids are typically discarded (Rabaglia et al 2019; John Crowe, USDA Animal and Plant Health Inspection Service, personal communication; Troy Kimoto, Canadian Food Inspection Agency, personal communication). The results of this study suggest that surveillance programs could gain additional useful information about fungal communities by screening insect trap fluids. Additionally, this could promote closer connections between entomologists and pathologists involved in forest health research and monitoring, as recently advocated by Jactel et al. (2020). Future studies should test the method at additional sites and also test

whether the efficacy of fungal pathogen detection is affected by the type of liquid preservative used. In addition, future work should conduct detailed comparisons of fungal communities sampled by different survey tools to determine which tools are optimal for which taxa.

Declarations

Conflict of interest: The authors declare no conflict of interest.

Author contributions

Research conceived by JAB, JA and JS. JAB and PNG conducted molecular analyses. JA, KR, CH and JS collected and processed samples and JS conducted statistical analyses. JA, JAB and JS wrote the manuscript. All authors approved the final version of the manuscript.

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Compliance with Ethical Standards

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Tables

Table 1. Number (%) of OTUs of all fungi, plant pathogens, and forest pathogens detected at each site by trap fluids (TF) versus aerial spore collectors (ASC). Heterogeneity chi square tests indicated significant differences among sites in proportion of species detected by trap fluids vs. aerial spore collectors for each category, i.e., all fungi, plant pathogens and forest pathogens (P< 0.0001).

Site	All fungi						Plant pathogens						Forest pathogens					
	total	TF	(%)	ASC	(%)	*P*	total	TF	(%)	ASC	(%)	*P*	total	TF	(%)	ASC	(%)	*P*
NB	896	692	77	546	61	<0.0001	159	122	77	117	74	0.604	77	63	82	54	70	0.130
NS	743	595	80	393	53	<0.0001	123	100	81	83	67	0.005	58	54	93	35	60	<0.0001
ON	848	760	90	388	46	<0.0001	176	167	95	89	51	<0.0001	78	73	94	35	45	<0.0001
Pooled sites	1277	1145	90	801	63	<0.0001	220	205	93	158	72	<0.0001	101	95	94	68	67	<0.0001

Table 2. Mean (± SE) abundance (DNA reads, log-transformed) of 25 of 101 forest pathogens captured in aerial spore collectors (ASC) vs. Lindgren funnel trap fluids (TF), in forest vs. urban sites in New Brunswick, Nova Scotia and Ontario, in 2018.

OTU	Species	Treatment					Site type					Treat* Site type
		ASC		TF			Forest		Urban			
		mean	SE	mean	SE	*P*	mean	SE	mean	SE	*P*	*P*
OTU_3	*Ceratocystis* sp. strain ML3 18S ribosoma	3.83	0.15	2.82	0.46	0.04	3.56	0.25	3.08	0.46	0.16	0.57
OTU_651	*Cytospora* sp. VL218 18S ribosomal RNA ge	0.66	0.30	1.80	0.28	0.06	1.49	0.35	0.96	0.30	0.03	0.39
OTU_210	*Exobasidium canadense* strain CGMCC 5.164	1.00	0.31	1.65	0.38	0.53	1.63	0.38	1.02	0.31	0.11	0.27
OTU_46	*Ganoderma applanatum* strain AMB04 small	2.32	0.33	2.84	0.23	0.37	2.88	0.14	2.28	0.38	0.37	0.69
OTU_291	*Ganoderma tsugae* voucher CFMR:DLL2011-26	0.85	0.32	1.64	0.33	0.62	1.27	0.34	1.22	0.35	0.22	0.08
OTU_469	*Hyphoderma setigerum* voucher CFMR:DLL201	1.80	0.27	1.19	0.28	0.14	1.76	0.25	1.23	0.29	0.19	0.20
OTU_585	*Mycosphaerella fimbriata* CBS 120736 ITS	0.48	0.25	1.23	0.36	0.30	1.15	0.38	0.57	0.24	0.17	0.62
OTU_86	*Mycosphaerella* sp. strain SO1_T26_L3B sm	2.73	0.28	1.61	0.31	<0.01	1.90	0.37	2.44	0.27	0.20	0.47
OTU_966	*Ophiognomonia michiganensis* strain CBS 1	0.79	0.25	0.76	0.24	0.84	0.99	0.27	0.56	0.20	0.27	0.85
OTU_54	*Ophiostoma* sp. WIN(M) 1634 18S ribosomal	0.00	0.00	1.98	0.48	<0.0001	1.19	0.52	0.79	0.36	0.76	<0.0001
OTU_111	*Phlyctema vagabunda* strain S1_T35_L4A_1	0.66	0.29	1.64	0.44	<0.01	1.37	0.44	0.93	0.35	0.61	0.84
OTU_27	*Phyllactinia guttata* isolate 4_153 small	1.68	0.31	2.67	0.33	<0.001	2.01	0.40	2.34	0.30	0.20	0.78
OTU_127	*Ramularia vizellae* strain OA1I5Mb small	1.05	0.39	1.06	0.47	0.18	0.64	0.34	1.47	0.46	0.56	0.47
OTU_103	*Rhizosphaera kalkhoffii* isolate gab0501	0.74	0.40	1.25	0.38	0.89	1.57	0.46	0.43	0.24	0.05	0.29
OTU_177	*Rhizosphaera* sp. JYP-2008 strain P02011	1.24	0.41	0.89	0.34	0.36	0.96	0.31	1.18	0.43	0.34	0.02
OTU_29	*Sydowia polyspora* strain VL187M 18S ribo	1.72	0.48	2.74	0.22	0.82	2.67	0.33	1.80	0.42	0.13	0.99
OTU_37	*Taphrina carpini* PYCC 5558 ITS region; f	1.55	0.34	2.79	0.19	0.01	2.13	0.33	2.22	0.34	0.95	0.87
OTU_299	*Taphrina communis* strain NRRL T-842 inte	0.67	0.26	1.90	0.26	0.02	1.13	0.27	1.44	0.35	0.40	0.70
OTU_165	*Taphrina dearnessii* strain NRRL T-796 in	0.83	0.31	2.26	0.27	0.03	1.61	0.31	1.48	0.40	0.76	0.63
OTU_87	*Taphrina flavorubra* strain NRRL Y-17795	1.23	0.34	2.61	0.24	0.01	1.85	0.37	2.00	0.34	0.23	0.47
OTU_66	*Taphrina johansonii* strain TJ001 18S rib	0.49	0.26	1.92	0.42	0.001	1.54	0.45	0.86	0.34	0.08	0.40
OTU_255	*Taphrina nana* strain CBS 336.55 internal	0.35	0.24	1.23	0.40	0.2	0.86	0.32	0.73	0.39	0.07	0.73
OTU_101	*Taphrina sacchari* strain NRRL T-210 inte	0.74	0.27	2.16	0.38	<0.001	1.72	0.37	1.18	0.39	0.02	0.09
OTU601921	*Taphrina* sp. isolate PCL9 18S ribosomal	0.29	0.20	1.79	0.49	0.01	1.29	0.49	0.79	0.36	0.08	0.77
OTU_1026	*Taphrina virginica* 046.1c internal trans	0.67	0.25	1.48	0.25	<0.01	1.41	0.28	0.74	0.24	<0.001	0.03

Figures

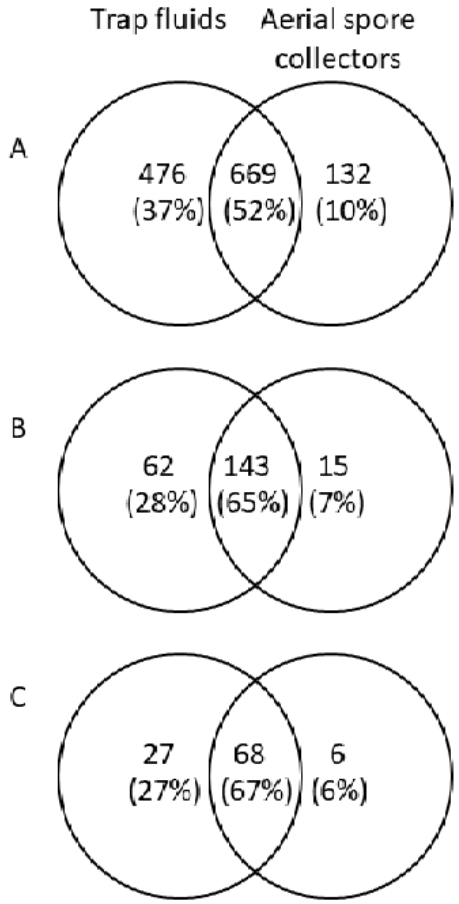

Figure 1

Venn diagrams showing the numbers of fungal OTUs detected by trap fluids and aerial spore collectors in 2018 at forest and urban sites in New Brunswick, Nova Scotia, and Ontario (data pooled from all sites) of: A) total fungi; B) plant pathogens; and C) forest pathogens.

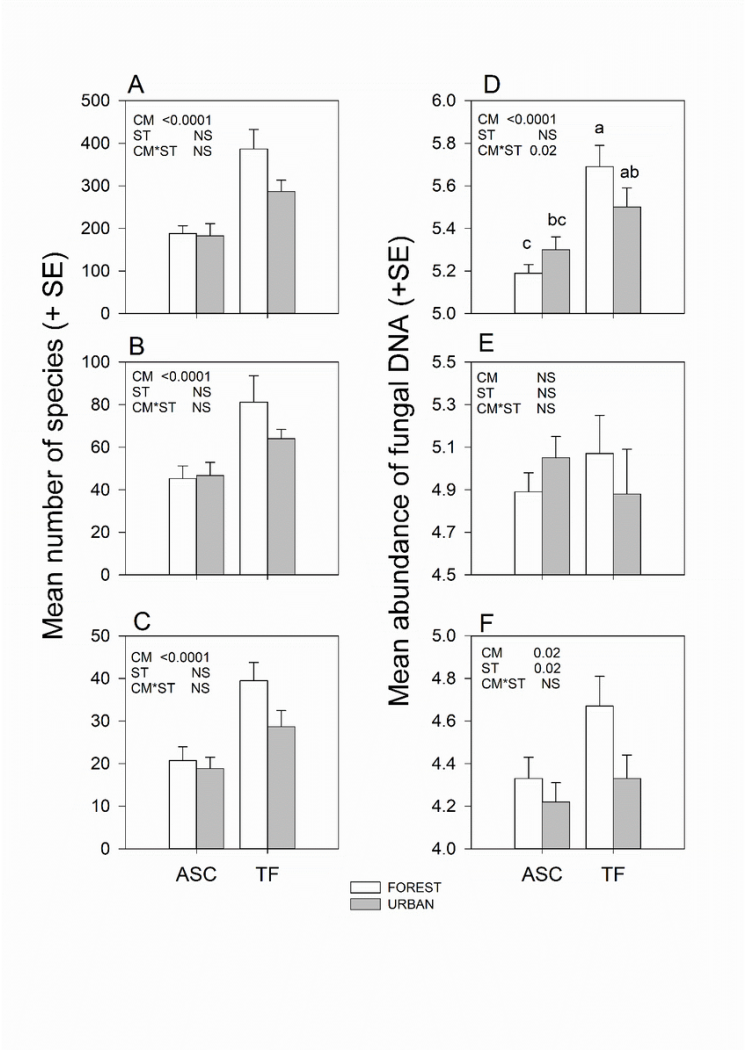

Figure 2

Mean (+SE) species richness (A, B, C) and mean (+SE) abundance of DNA (D, E, F) of all fungi (top), plant pathogens (middle), and forest pathogens (bottom) collected by aerial spore collectors (ASC) vs. trap fluids (TF) in forest and urban sites in New Brunswick, Nova Scotia, and Ontario, during a 4 week period in August-September 2018. Significance of collection method (CM), site type (ST) and their interaction (CM*ST) from generalized linear mixed models. Tukey test on least square means was performed only on mean abundance of fungi for collection method-site type combinations (D) due to the significant interaction; means followed by diferent letter were significantly different ($P \leq 0.05$).

Supplementary Files

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