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Aerobiological sampling efficiency of media-containing Petri plates for use in lower atmosphere spore collection

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Abstract Petri plates (PP) containing volumes of media attached to unmanned aircraft systems (UAS) have been used to trap atmospheric biota for years. If capture efficiency varies based on media volume used, aerial concentrations of biota may be poorly estimated after an UAS flight occurs. We conducted 36 separate sampling flights in which PP were filled either with media to maximum volume or half the maximum volume to determine efficiency of each in the collection of viable spores from the genus *Fusarium* in the lower atmosphere. Overall, the media at maximum volume collected 56 % more spores than the media at half-volume when analyzing the average collection for all flights. In research that relies on quantification of an aerial biota source, UAS equipped with media-containing plates filled with less than maximum volume will hinder spore collection efficiency.

Keywords *Fusarium* · Aerial sampling · Unmanned aircraft systems · Spore capture

1 Introduction

Aerobiology is the study of factors and processes that influence the movement of biota in the atmosphere and

involves biota liberation from inoculum sources, horizontal transport, deposition, and the ecological and environmental factors that contribute to each (Isard and Gage 2001). The ability of biota to move long distances is essential to their evolutionary survival. Atmospheric movement may result in the invasion of new sites, utilization of new resources, and gene transfer among populations (Rabb 1985). It can also result in the introduction of a pathogen to a region or continent or the frequent re-introduction of a pathogen to a region (Aylor 1986). Recent aerial sampling in the middle-to-upper troposphere (8–15 km altitude) indicates microbial communities exist including bacteria and fungi that are available for long-range transcontinental transport (DeLeon-Rodriguez et al. 2013). These findings are in addition to the existing knowledge of biota transport within the lower 3 km of the earth's atmosphere.

Methods of trapping atmospheric biota using aircraft have varied over the years. Stakman et al. (1923) used Vaseline-smeared microscope slides exposed by hand and mechanical spore traps on aircraft to capture fungal spores. Kelly et al. (1951) described several methods including the use of a B-29 aircraft and electrostatic sampling devices by which bacteria and fungi can be collected from the atmosphere. Unmanned aircraft systems (UAS) have been used more recently to collect various biota. Dauer et al. (2009) and Shields et al. (2006) used UAS to collect aerially dispersed seeds of *Conyza canadensis* (horseweed) to determine the possible transfer of

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glyphosate resistance genes. Aylor et al. (2006) used pollen samples collected with UAS to predict gene flow by quantifying aerial pollen concentrations. Insect nets secured to UAS have been used to collect *Empoasca fabae* (potato leafhopper) to determine initiation events of fall migration (Shields and Testa 1999). Sampling devices with media-containing Petri plates (PP) attached to UAS have been used to collect *Phytophthora infestans* sporangia (Aylor et al. 2011) and to collect *Fusarium* ascospores (Maldonado-Ramirez et al. 2005; Schmale et al. 2012).

In 2012, the Federal Aviation Administration (FAA) reauthorization bill passed by the U.S. Congress compelled the FAA to integrate civil UAS into U.S. airspace (FAA 2012). The resulting rules will define UAS-specific restrictions, pilot certification and licensing, and other safety precautions involving registration and operational limitations. As of February 2013, 327 active Certificates of Authorization were registered through the FAA (Anonymous 2013), suggesting numerous UAS flights for various purposes in the lower atmosphere including those for research purposes may increase in years to come.

Fusarium spp. were chosen as the model organisms for this aerial sampling project due to their presence in the planetary boundary layer (PBL) at all hours of the day and night and through much of the growing season (Maldonado-Ramirez et al. 2005). PP filled to maximum volume assisted in the collection of approximately 13,000 colony-forming units (CFU) during 158 UAS flights in May and June of 1999–2002 (Maldonado-Ramirez et al. 2005). Much lower CFU counts than reported by Maldonado-Ramirez et al. (2005) have been recently reported (Lin et al. 2013; Schmale et al. 2012) suggesting the media volume in PP directly affects quantity of aerial spore collection.

To determine the effect media volume in PP has on spore collection efficiency, we conducted 36 flights in 12 flight periods over 3 days with 2 different media volumes.

2 Materials and methods

2.1 Collection of *Fusarium* spp. with unmanned aircraft systems

Sampling devices attached to UAS were used to collect viable spores of the genus *Fusarium* from the

lower atmosphere above Cornell University's Musgrave Research Farm in Aurora, New York (Fig. 1). Twelve flight periods were performed during daylight hours (9 a.m. to 1 p.m.) from September 10–12, 2012. Three UAS were deployed simultaneously and flown at approximately the same altitude for a total of 36 flights. The UAS were radio-controlled airplanes with a 3.0 m wingspan and powered with a 50 cm³ engine (Aylor et al. 2011; Shields et al. 2006). Each UAS was equipped with a real-time telemetry consisting of a GPS antenna (Byonics GPS2TM), low power transmitter (Yaesu VX-177TM), and data module (Byonics TinyTrak3TM). Each ground-based receiving station consisted of an antenna (Cushcraft AR-270TM), RF matching receiver (Yaesu VX-177TM), data receiving module (Byonics TinyTrak4TM), and a laptop where the data was stored. Data packets were received every 2 s from the UAS. Telemetry data included Universal time, ground speed, altitude, and current position (latitude, longitude). Data were recorded individually for each UAS on three dedicated down-links. The GPS information was used to plot UAS paths and altitudes during each sampling period.

Each UAS was equipped with two spore sampling devices; one mounted under the left wing and one mounted under the right wing (Fig. 1). Each sampling device had two arms, and each sampling arm was attached to a large sail servo (Model 94581, Airtronics Inc., Anaheim, CA), allowing the sampling arms to be moved from the protected takeoff position to the sampling position remotely when the UAS had reached the predetermined sampling altitude. Each arm on each sampling device held a single full-volume PP, a single half-volume PP, and a single grease PP. All three types of PP were vertically mounted on the arms. Each sampling device had two arms, and two sampling devices were mounted on each UAS resulting in 4 full-volume, 4 half-volume, and 4 grease PP per UAS per flight. The full- and half-volume PP (60 mm × 15 mm; Falcon 351007, Becton Dickson Labware, Franklin Lakes, NJ) contained a *Fusarium*-selective medium (FSM). The dimensions for area where media can be stored within PP were approximately 50 mm × 12 mm. The FSM included a modified Nash–Snyder medium prepared as described by Burgess et al. (1994) with an increased level of neomycin sulfate (Schmale and Bergstrom 2004). PP were filled to full-volume (25 mL to an approximate 12 mm depth) and half-volume (12.5 mL to an approximate 6 mm depth). Due



Fig. 1 Unmanned aerial vehicles (UAS) were used to collect colony-forming units of *Fusarium* spp. in the planetary boundary layer. UAS had a 3.0 m wingspan and were powered with a 50 cm³ engine (a). Spore sampling devices were attached

to the *left* and *right* wings and were closed (b) and opened (c) remotely from the ground once aloft. PP of *Fusarium*-selective media at half-volume (d) and full-volume (e) were vertically mounted on sampling devices

to the full-volume media's close proximity to the lid of the PP, spacers were glued to the lid of each PP to prevent the media sampling surface from contacting the lid. The bottom of the grease PP was coated with a thin layer of silicon grease (SDI, Plymouth Meeting, PA). The silicon grease was dissolved in hexane for 3 h (3.3 g silicone grease per 30 mL of hexane) (Aylor et al. 2011). All PP remained closed and were stored in containers for transport to the field and remained this way until placed onto sampling devices. Lids of PP remained closed while loading sampling devices and were removed only when sampling devices were closed prior to takeoff.

Sampling devices were opened remotely from the ground once UAS reached sampling altitude and were closed after 15 min and before descent. The sampling surface was oriented so that the media or grease was

perpendicular to the airflow when in operation. When the sampling devices closed, both arms were rotated forward parallel to the direction of travel, and the sampling surfaces of the PP were protected from exposure. When open, the arms rotated to a position perpendicular to the direction of travel, and the sampling surfaces of the PP were exposed to the air stream. Temperature and precipitation were recorded by a weather station located at the Musgrave Research Farm during the flight periods.

After flight periods were completed, full- and half-volume PP were removed from each UAS and incubated for 5 days in the laboratory at ambient room temperature. Individual CFU of mycelia characteristic of *Fusarium* spp. (Leslie and Summerell 2006) were counted and recorded for PP from each

plane and flight period (Maldonado-Ramirez et al. 2005). Grease PP were removed from each UAS and were taken to the laboratory where the grease surface was stained with lactophenol cotton blue (Sigma-Aldrich, Inc., Allentown, PA), and a disk of light acetate was placed on top allowing *Fusarium* spp. spores to be distinguished at 100 $\times$ magnification from spores and other material captured on the grease.

2.2 Statistical analyses

We tested for significant differences in CFU among full and half PP, among planes, among days, among sampling times (8 a.m. to 1 p.m.), among position on plane wings, and between the left and right side of each plane. Statistical analyses were performed using the generalized linear mixed model (JMP Pro 10.0.0, SAS Institute Inc., Cary, NC) to allow for both fixed and random effects. The random effect included in the model accounted for the specific location of each plate on either the left or the right wing of each UAS. If significant differences were found, means were separated using Tukey–Kramer HSD test. Significance was evaluated at $P \leq 0.01$ for all tests. Data from CFU were transformed to ensure normal distribution before analysis.

3 Results

Flight speeds during sampling were between 21.1 and 22.8 m s⁻¹ (avg. 21.5 $\pm$ 0.7 m s⁻¹). Altitudes averaged between 34.4 and 70.4 m (avg. 49.3 $\pm$ 8.0 m) above ground level, and each PP was exposed to approximately ~ 2.6 m³ of air per minute at UAS flight speeds of 21.5 m s⁻¹. This result is based on calculations made by Aylor et al. (2011) for the collection of a similar particle size (~ 20 μ) using UAS. Particle collection efficiency, E_{pp} , of the PP is described by Aylor et al. (2006), and we calculated the efficiency of capture to be approximately 0.08 (8 %) for full-volume PP based on the ~ 0.002 m s⁻¹ settling velocity in still air of ascospores (Schmale et al. 2005). Half-volume PP were approximately 56 % less efficient than the full-volume PP based on mean CFU counts (Fig. 2).

Viable spores of *Fusarium* spp. were present during every hour of the flight periods. We collected a total of 35,446 CFU of *Fusarium* spp. during 36 flights in 12

flight periods over 3 days. CFU counts ranged from 64 to 500 for full-volume PP and from 0 to 341 for half-volume PP. The mean CFU count for all flight periods was 213.07 $\pm$ 93.32 for the full-volume PP and 62.19 $\pm$ 73.01 for the half-volume PP. The full-volume PP collected 56 % more than the half-volume PP when analyzing the totals for all 12 flight periods. Collection on grease PP ranged from 0 to 6 *Fusarium* spores per plate.

Temperatures on 10 September, 11 September, and 12 September ranged from 8.3 to 20.6 $^{\circ}$ C (avg. 14.4 $^{\circ}$ C), 6.7 to 18.3 $^{\circ}$ C (avg. 15.6 $^{\circ}$ C), and 11.7 to 26.7 $^{\circ}$ C (avg. 19.4 $^{\circ}$ C), respectively. No precipitation was recorded for the three sampling days. During flight periods on September 10, September 11, and September 12, 2012, mean CFU counts on full-volume PP were 214.78 $\pm$ 81.01, 153.86 $\pm$ 40.44, and 259.21 $\pm$ 104.35, respectively. Mean CFU counts on half-volume PP were 23.5 $\pm$ 43.01, 41.09 $\pm$ 21.68, and 98.11 $\pm$ 92.73, respectively. CFU counts for each flight period are described in Fig. 2.

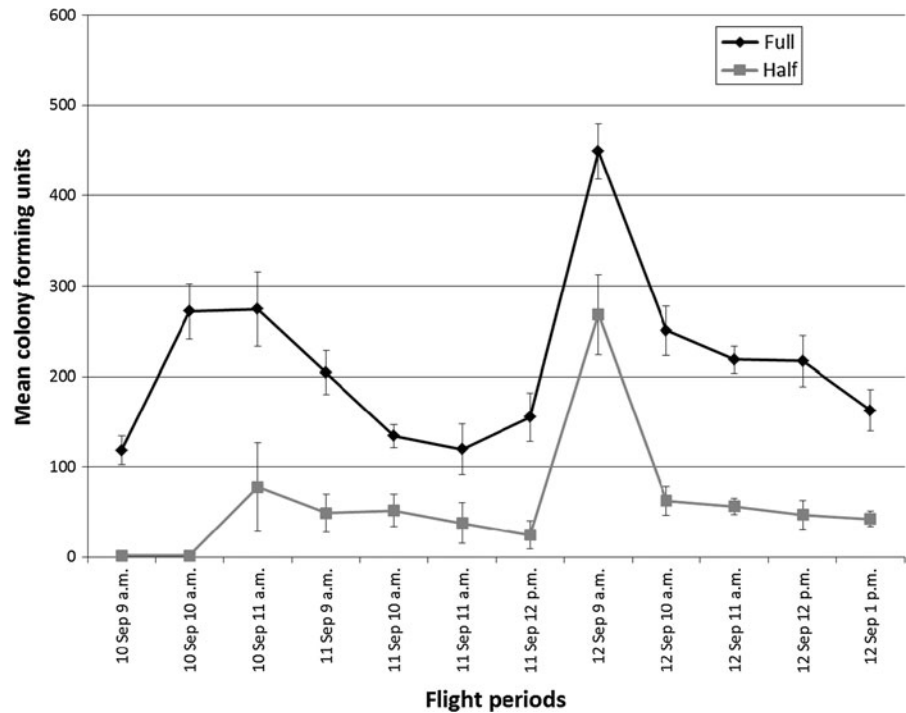
There was a significant difference between mean CFU counts on the total full-volume and the total half-volume PP ($P \leq 0.0001$). There was also a significant difference in mean CFU counts among sampling days ($P \leq 0.0001$) but not hours ($P = 0.5520$). The mean CFU counts on 10 September and 11 September were significantly different from those on 12 September ($P = 0.0003$). No significant difference was observed for mean CFU counts of PP located at different positions on the left and right side of each plane ($P = 0.5375$) or for different planes used ($P = 0.0512$).

The amount of water lost from the media of both full- and half-volume PP was calculated by measuring the weight of each plate prior to takeoff and after each flight. Average water loss was 9 % (3.30 $\pm$ 0.8 g) from full-volume PP and 1 % (0.42 $\pm$ 0.3 g) from half-volume PP. Water lost from full PP was significantly higher than half-volume PP ($P < 0.0001$).

4 Discussion

Viable spores of *Fusarium* were present in the PBL during all of the flight periods, and significant differences in average CFU collection were observed. The turbulent nature of the PBL during these particular daylight hours may explain these observations. Atmospheric turbulence affects the ascent of spores before

Fig. 2 Colony-forming units of *Fusarium* spp. were collected with unmanned aerial vehicles in the lower atmosphere. Each flight period included 3 planes equipped with spore sampling devices flown simultaneously over agricultural fields in Aurora, New York. Each sampling device consisted of PP with *Fusarium*-selective media at full-volume and half-volume



they become airborne (Pedgley 1985). Vertical mixing occurs in the PBL when the sun heats the surface of the earth, warming the air in contact with the surface. Warm air then rises carrying biota from near the surface aloft. Cooler air replaces the rising warm air to be subsequently warmed with contact to the earth's surface. Solar radiation and moderate wind speeds increase daytime convection while nocturnal cooling of surface air inhibits convection in the atmosphere (Sparks et al. 1985). Thermal convection promotes the mixing of surface air and spores with the air aloft (Sparks et al. 1985). Spores may be carried aloft to heights of several km through a succession of updrafts. These updrafts create a mixing layer which can be greater in the afternoon and warmer months and can be the shallowest on calm nights (Pedgley 1985). Organisms may successfully escape the turbulence found near the surface of the earth into the PBL where higher wind speeds and laminar-like air flow disperse the biota long distances (Cox 1987). The altitudes of the UAS averaged approximately 50 m above ground level; therefore, the spore collection may have occurred below the highest aerial concentration (i.e., 1–3 km) of *Fusarium* spores during the day (Maldonado-Ramirez et al. 2005).

The importance of sampler efficiency varies with the focus of the aerial flight. If the goal of the flight is

to only collect biota present in the atmosphere, then a highly efficient sampling surface may not be as important and a less efficient sampling surface may actually be advantageous because the sampling surface may not become saturated during the duration of the flight. If targeted detection of specific biota is the focus of the flight, a highly efficient surface is only advantageous when the aerial density of the target organism is low. However, flights focused on quantifying the aerial densities of biota require an efficient sampling surface and an accurate estimate of the actual sampling efficiency.

Our data show that a sampling surface with no edge to inhibit airflow across the surface is more than two times more efficient than a surface with a 6 mm edge. More importantly, the comparative efficiency is not consistent and changes with different atmospheric and biota density conditions. Our collection of 35,000 CFU of *Fusarium* spp. is much higher than the 13,000 CFU originally collected with full-volume PP used by Maldonado-Ramirez et al. (2005). However, Maldonado-Ramirez et al. (2005) focused on the collection of one species (e.g., *Fusarium graminearum*) and did not quantify other *Fusarium* spp. as seen in our collection efforts.

In all 12 flight periods, full-volume PP were able to consistently collect more CFU than half-volume PP

(Fig. 2). Filling PP with media to any volume less than completely full, creates an edge, or physical barrier, on all sides of PP. Biota-containing air spills over PP as it deposits the biota to the media, but when the air encounters an edge, such as with less-than-full-volume PP, the airflow differs. Many of the half-volume PP had spores adhering to the inside of the edge, suggesting a less-than-smooth airflow across the plate and a zone of turbulence just inside the edge. In contrast, full-volume PP were able to uniformly collect CFU no matter the PP location on the sampler or whether on a particular wing. Research using half-volume PP to sample spores in the atmosphere would not be accurate if estimating the CFU collection potential of full-volume PPs. This is due to the high percentage of variability between CFU collected in the full- and half-volume PP.

The capture (i.e., impaction) efficiency of airborne sampling devices for small particles can vary widely depending upon the settling velocity (v_s) of the target particle. Ascospores of *Venturia inaequalis* are reported to have a v_s of 0.002 m/s in still air (Aylor 1998) similar to what has been calculated for *Fusarium* ascospores. Sporangia of *P. infestans* have a reported v_s of 0.01 m/s in still air and a capture efficiency of 0.5 (50 %) with the use of 60 mm $\times$ 15 mm PP (Aylor et al. 2011). A much larger particle such as maize pollen has a v_s of 0.28 m/s and a capture efficiency of 0.97 (97 %) with 100 mm $\times$ 15 mm PP (Aylor et al. 2006). Water lost by PP in flight may also indicate differences in capture efficiency of PP. Significantly less water was lost by half-volume PP suggesting a difference in air movement across the PP compared to that of the air movement across the full-volume PP.

The capture efficiency of *Fusarium* ascospores on 60 mm $\times$ 15 mm PP was originally estimated to be 20 % (Maldonado-Ramirez et al. 2005; Shields and Testa 1999); however, we now have a more accurate v_s for *Fusarium* resulting in lower efficiency than previously reported. We calculated the capture efficiency of full-volume PP to be 8 % based on the v_s of *Fusarium* ascospores. Half-volume PP can be estimated to be less efficient (e.g., approximately 2–4 %) than full-volume PP based on our results. Although grease PP should also have a capture efficiency of 8 %, we observed a much lower retention of *Fusarium* spores during the flight periods although many more spores were available in the lower atmosphere based on CFU counts from full- and half-volume PP. Previous research has reported

0–148 *Fusarium* spores per grease PP (Maldonado-Ramirez et al. 2005). One possibility for the low number of spores captured is the thickness of the grease layer distributed to each PP. If the grease did not retain the 20 μ *Fusarium* spore, it is possible numerous spores impacted the surface but did not adhere to the grease. Another possibility may be the lack of adhesive properties of the grease due to temperature or humidity levels, but additional flights with grease PP are needed before further conclusions can be made.

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