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Seasonal associations and atmospheric transport distances of fungi in the genus *Fusarium* collected with unmanned aerial vehicles and ground-based sampling devices

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HIGHLIGHTS

- *Fusarium* was collected with autonomous UAVs and ground-based sampling devices.
- 2218 colony forming units (CFUs) of *Fusarium* were collected from 2009 to 2012.
- Spore concentrations were higher in the fall, spring, and summer, and lower in the winter.
- Samples collected during the winter were likely coming from more distant sources.
- Knowledge of aerobiology of *Fusarium* could inform air pollution and disease spread.

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ABSTRACT

Spores of fungi in the genus *Fusarium* may be transported through the atmosphere over long distances. New information is needed to characterize seasonal trends in atmospheric loads of *Fusarium* and to pinpoint the source(s) of inoculum at both local (farm) and regional (state or country) scales. We hypothesized that (1) atmospheric concentrations of *Fusarium* spores in an agricultural ecosystem vary with height and season and (2) transport distances from potential inoculum source(s) vary with season. To test these hypotheses, spores of *Fusarium* were collected from the atmosphere in an agricultural ecosystem in Blacksburg, VA, USA using a Burkard volumetric sampler (BVS) 1 m above ground level and autonomous unmanned aerial vehicles (UAVs) 100 m above ground level. More than 2200 colony forming units (CFUs) of *Fusarium* were collected during 104 BVS sampling periods and 180 UAV sampling periods over four calendar years (2009–2012). Spore concentrations ranged from 0 to 13 and 0 to 23 spores m^{-3} for the BVS and the UAVs, respectively. Spore concentrations were generally higher in the fall, spring, and summer, and lower in the winter. Spore concentrations from the BVS were generally higher than those from the UAVs for both seasonal and hourly collections. A Gaussian plume transport model was used to estimate distances to the potential inoculum source(s) by season, and produced mean transport distances of 1.4 km for the spring, 1.7 km for the summer, 1.2 km for the fall, and 4.1 km for the winter. Environmental signatures that predict atmospheric loads of *Fusarium* could inform disease spread, air pollution, and climate change.

1. Introduction

Fungi in the genus *Fusarium* cause devastating diseases in plants, domestic animals, and humans (Berek et al., 2001; Bush et al., 2004; Leslie and Summerell, 2006; McMullen et al., 1997). These fungi produce a wide range of spore types (microconidia, mesoconidia, macroconidia, and ascospores) that may be transported through the atmosphere (Fernando et al., 2000; Katan et al., 1997; Schmale et al., 2005). New information is needed to characterize seasonal trends in atmospheric loads of *Fusarium* and to pinpoint the source(s) of inoculum at both local (farm) and regional (state or country) scales. Such knowledge could assist in predicting the movement of these fungi and contribute to early warning systems for disease (BozorgMagham et al., 2013; Strange and Scott, 2005; Tallapragada et al., 2011).

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Autonomous unmanned aerial vehicles (UAVs) have been developed to collect spores of *Fusarium* in the lower atmosphere (Schmale et al., 2008). These UAVs have been used to characterize fluctuations in colony forming units (CFUs) of *Fusarium* over short time intervals (Lin et al., 2013), and to establish that isolates of single species of *Fusarium* (*F. graminearum*) collected 40–320 m above ground level cause disease and produce mycotoxins (Schmale et al., 2012). Ground-based technologies have also been developed to collected microbes near the surface of the earth, such as the Burkard volumetric sampler (BVS) (Kennedy et al., 2000). Here, we leverage technologies with autonomous UAVs and a BVS to examine associations between CFUs of *Fusarium* collected 1 m and 100 m above the ground. Previous work has shown that spore concentrations generally decrease with increasing height above ground level (Aylor, 1998; Dasgupta, 1988; de Jong et al., 2002; Khattab and Levetin, 2008). We hypothesized that (1) atmospheric concentrations of *Fusarium* spores vary with height and season and (2) transport distances from potential inoculum source(s) vary with season. To test these hypotheses, *Fusarium* spores were collected from the atmosphere using a BVS 1 m above ground level and UAVs 100 m above ground level. These collections were performed across multiple seasons over four years (where we consider meteorological seasons). The specific objectives of this work were to (1) examine seasonal associations between atmospheric concentrations of *Fusarium* spores 1 m and 100 m above the ground in an agricultural ecosystem and (2) compute potential transport distances of fusaria from their hypothesized source(s). An increased understanding of the aerobiology of *Fusarium* may contribute to new and improved control strategies for plant diseases (Aylor, 1998), inform predictions of the movement of allergens from natural and managed ecosystems (Sadyś et al., 2014), and improve our knowledge of factors influencing air pollution and climate change (Frenguelli, 2013; Morris et al., 2011).

2. Materials and methods

2.1. Sample collection procedures

Two sampling devices were used in this study: an autonomous (self-controlling) UAV (Schmale et al., 2008) and a BVS (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England) (Kennedy et al., 2000). The BVS was used to collect *Fusarium* 1 m above ground level (AGL) for target sampling intervals of about 120 min, and the UAVs were used to collect *Fusarium* 100 m AGL for target sampling intervals of about 10 min (Fig. 1). Most of the UAV samples were collected within a Burkard sampling interval, such that both samplers were operating simultaneously and thus the resulting collections could be compared (Fig. 1). Each sampling device contained large (9 cm diameter) plates of *Fusarium* selective medium (FSM) as described by Schmale et al., 2006 and Lin et al., 2013. All samples were collected at Virginia Tech's Kentland Farm in Blacksburg, VA, USA across multiple seasons over four calendar years (2009, 2010, 2011, and 2012). Seasons were defined as the following: spring (1 March–31 May), summer (1 June–31 August), fall (1 September–30 November), and winter (1 December–28 February).

2.2. Culturing and identification of Fusarium

Colonies of *Fusarium* were cultured and identified as described by Lin et al. (2013). Following each sampling mission, sampling plates were returned to the laboratory and incubated for 7–15 days at ambient room temperature. White, fluffy colonies of *Fusarium* were counted on each of the sampling plates. Each individual colony of *Fusarium* was subcultured onto new plates of FSM and single-spored into small plates of ¼-strength potato dextrose agar for downstream identification and cryogenic storage.

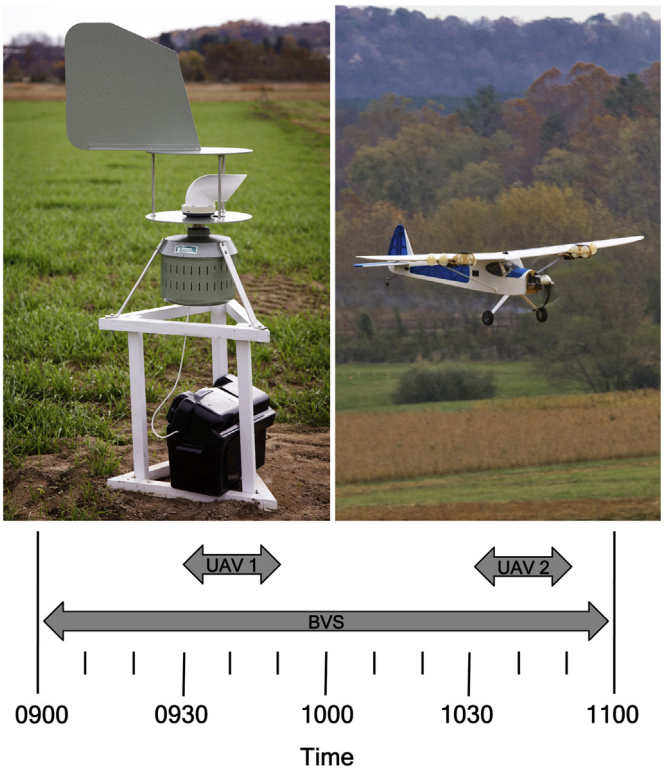

Fig. 1. Illustration of sampling plan for *Fusarium* using a Burkard volumetric sampler (BVS) 1 m above ground level (left) and an unmanned aerial vehicle (UAV) 100 m above ground level (right). The BVS collected samples for 120 min, and the UAVs collected samples for 20 min. Both samplers were operated concurrently.

2.3. Collection efficiencies of UAVs and BVS

Spore concentrations were divided by collection efficiencies of UAVs and the BVS to get corrected aerial spore concentrations. Collection efficiencies were calculated using published methods (Aylor, 1993; Aylor et al., 2006; Chamberlain, 1975; McCartney et al., 1993). Spore sizes were determined for *Fusarium* species previously identified in the atmospheric assemblages (Schmale, unpublished observations) by measuring the length and width of three randomly selected spores as described by Leslie and Summerell (2006) (Table 1). This information was used to calculate the aerodynamical diameter, d_a, of each spore described as (Chamberlain, 1975; McCartney et al., 1993),

$$d_a = 1.145\left(\frac{1}{\alpha}\right)^{\frac{1}{2}} s_d^{\frac{2}{3}} s_l^{\frac{1}{3}} \quad (1)$$

$$\alpha = 0.087\left(\frac{s_l}{s_d}\right) + 0.97 \quad (2)$$

where s_d is the spore diameter and s_l is the spore length. The d_a of each spore was used to estimate the settling velocity, v_s, which was calculated as a function of Stoke's drag defined by Chamberlain (1975) and McCartney et al. (1993),

$$v_s = \frac{g d_a^2 (\rho_p - \rho_a)}{18\mu} \quad (3)$$

where g is the gravitational acceleration (9.8 m s^{-2}), μ is the dynamic viscosity of the air (1.81×10^{-5} kg m^{-1} s^{-1} at STP), ρ_p is the

Table 1
Estimates of collection efficiencies with unmanned aerial vehicles (UAVs) and a Burkard volumetric sampler (BVS) for different spore types (macroconidia, mesoconidia, microconidia, and ascospores) from 12 species of *Fusarium*. The species listed were among those tentatively identified from 11 different UAV flights (Schmale et al., unpublished observations).

Species	Spore type	Spore diameter (μm)[a]	Spore length (μm)[a]	d_a (μm)[b]	v_s (mm/s)[c]	E_{pp}^{UAV}[d]	E_{pp}^{BVS}[e]
F. armeniacum	Macroconidium	2.9	56	5.47	0.90	1.34%	99.98%
F. avenaceaum	Macroconidium	2.9	59	5.48	0.90	1.35%	99.98%
	Ascospore	4.5	16	6.95	1.45	2.75%	99.97%
F. circinatum	Macroconidium	2.9	29	5.27	0.84	1.20%	99.98%
	Microconidium	2.9	9	4.35	0.57	0.67%	99.99%
	Ascospore	5.0	14	7.32	1.61	3.21%	99.96%
F. equiseti	Macroconidium	1.5	85	2.72	0.22	0.16%	99.99%
	Ascospore	5.0	27	8.37	2.11	4.75%	99.95%
F. fujikuroi	Macroconidium	2.9	41	5.41	0.88	1.30%	99.98%
	Microconidium	2.9	15	4.82	0.70	0.92%	99.98%
	Ascospore	5.0	15	7.44	1.67	3.37%	99.96%
F. graminearum	Macroconidium	4.4	56	8.16	2.00	4.41%	99.95%
	Ascospore	3.5	21	5.96	1.07	1.74%	99.98%
F. nygamai	Macroconidium	5.9	71	10.90	3.57	10.05%	99.92%
	Microconidium	5.5	14	7.88	1.86	3.98%	99.96%
F. oxysporum	Macroconidium	3.7	44	6.83	1.40	2.61%	99.97%
	Microconidium	2.9	9	4.35	0.57	0.67%	99.99%
F. proliferatum	Macroconidium	2.9	41	5.41	0.88	1.30%	99.98%
	Microconidium	2.2	6	3.20	0.31	0.26%	99.99%
	Ascospore	5.0	16	7.55	1.71	3.51%	99.96%
F. sambucinum	Macroconidium	5.1	44	9.13	2.51	6.10%	99.94%
	Ascospore	8.0	24	11.91	4.26	12.75%	99.90%
F. sporotrichioides	Macroconidium	2.9	32	5.32	0.85	1.24%	99.98%
	Mesoconidium	2.9	25	5.19	0.81	1.15%	99.98%
	Microconidium	4.4	7	5.59	0.94	1.43%	99.98%
F. verticillioides	Macroconidium	2.9	62	5.48	0.90	1.35%	99.98%
	Microconidium	2.9	10	4.45	0.60	0.72%	99.99%

[a] Spore dimensions (length and width) were estimated by measuring three spores for each species as listed in Leslie and Summerell (2006).
[b] Calculated using Eq. (1).
[c] Calculated using Eq. (3).
[d] Calculated using Eq. (4). The flight speed of the UAVs was 90 km h^{-1} and each sampling plate was 90 cm wide.
[e] Calculated using Eq. (7). The flow rate of the BVS was 20 L/min with an opening of 9 cm.

density of the spore (assumed to be 1000 kg m^{-3}), and ρ_a is density of the air (assumed to be 1.2 kg m^{-3}).

Finally, the sampling efficiency of the UAV, E_{pp}^{UAV}, was calculated as previously described (Aylor et al., 2006),

$$E_{pp}^{UAV} = \frac{0.99}{1 + 0.268 S_{UAV}^{-1.527}} \quad (4)$$

where the Stokes number relevant for UAV sampling, S_{UAV}, is given by:

$$S_{UAV} = \frac{U_0 \tau_R}{D_P} \quad (5)$$

where U_0 is the flight speed (Table 2), D_P is the diameter of the petri plate sampler, and τ_R is the particle relaxation time described by,

$$\tau_R = \frac{v_s}{g} \quad (6)$$

Sampling efficiency of the BVS was calculated as previously described (Aylor, 1993),

$$E_{pp}^{BVS} = 1 + \left[\left(\frac{U_a}{U_s}\right) - 1\right] f(S_{BVS}) \quad (7)$$

where U_a is the undisturbed airflow upwind of spore sampler (estimated to be ~0.1 m s^{-1} (Aylor, 1993)), U_s is the flow speed of the BVS (12 m s^{-1}), and $f(S_{BVS})$ is given by,

$$f(S_{BVS}) = \frac{2S_{BVS}}{(1 + 2S_{BVS})} \quad (8)$$

where the Stokes number relevant for BVS sampling, S_{BVS}, is given by,

$$S_{BVS} = \frac{v_s U_a}{gL} \quad (9)$$

where L is the width of the sample entrance on the BVS (estimated to be 9 cm). Table 1 shows the s_l, s_d, d_a, v_s, E_{pp}^{UAV}, and E_{pp}^{BVS} for representative species of *Fusarium* tentatively identified from some of the atmospheric samples (Schmale, unpublished observations). A digital anemometer (EA-3010U, La Crosse Technology, La Crosse, WI) was used to measure the sampling rate of the BVS in the laboratory at 21.5 L/min. Here, we retain the published sampling rate of the BVS as 20 L/min for our calculations.

Table 2
Parameters involved in estimating the aerial concentration and collection efficiency of *Fusarium* spores.

Parameter	Value	Source
g	9.8 m s^{-2}	Physical constant
μ	1.81×10^{-5} kg m^{-1} s^{-1}	Physical constant
ρ	1000 kg m^{-3}	(Chamberlain, 1975)
ρ_a	1.2 kg m^{-3}	Physical constant
U_0	90 km h^{-1}	UAV onboard measurement
D_P	9 cm	Diameter of petri dish
L	9 cm	Length of BVS opening
U_a	0.1 m s^{-1}	(Aylor, 1993)
U_s	12 m s^{-1}	(Aylor, 1993)
BVS flow rate	0.02 m^3 min^{-1}[a]	(Kennedy et al., 2000)
UAV flow rate	9.6 m^3 min^{-1}	(Aylor et al., 2011)
E_{eff}^{UAV}	≈1.34%	Derived parameter

[a] A digital anemometer (EA-3010U, La Crosse Technology, La Crosse, WI) was used to measure the sampling rate of the BVS in the laboratory at 21.5 L/min. Here, we retain the published sampling rate of 20 L/min for our calculations.

2.4. Calculations of spore concentrations

CFUs (viable spores) were converted to concentrations, C (CFU m^{-3}) (Aylor et al., 2011), from the number of spores collected on the samplers, N_P (colony forming units or CFUs), the volumetric flow rate of air sampling, V_R (m^3 min^{-1}), the efficiency of spore collection by the samplers, E_{pp}, and the duration of the sampling period, T_D (min) using,

$$C = \frac{C_{raw}}{E_{pp}} \tag{10}$$

where $C_{raw} = N_P/V_R T_D$ is the raw (uncorrected) concentration. The V_R of the sampling device is 0.02 m^3 min^{-1} for the BVS (a single sampling plate was used for each collection interval) and 9.6 m^3 min^{-1} per petri plate for the UAV (up to 8 sampling plates were used per flight), and T_D was 10–205 min for the BVS and 5–28 min for the UAV (Table 2). Since the *Fusarium* assemblages obtained via both the UAVs and BVS were not further resolved down to the species level in the present study, it was necessary to estimate an effective average efficiency for each method, E_{eff}^{UAV} and E_{eff}^{BVS}. To do this, we estimated collection efficiencies from 11 *Fusarium* assemblages (11 different UAV flights) that had been characterized down to the species level (Schmale, unpublished observations). We divided the uncorrected concentration C_{raw} for each flight population by the corrected concentration C (see Eq. (10), and obtained an effective efficiency for each flight. Taking the average efficiency for these 11 species-resolved flights, we obtain an average effective efficiency; essentially a weighted average of the UAV efficiencies given in Table 1.

2.5. Gaussian plume model

For the case of a steady, continuous release of spores from a point source at a height z_s above ground level, C can be represented by an equation for a Gaussian plume (Aylor, 1999), which varies with the height of the sampler, z according to

$$C \propto \exp\left(\frac{-(z-z_s)^2}{2\sigma_z^2}\right) \tag{11}$$

where $\sigma_z = ax^b$ is the effective height of the plume (in m) which changes with the horizontal downwind distance, x, of the sampler from the source and parameters a and b are a function of meteorological conditions (Prussin et al. unpublished observations). From relationship (11), we can obtain an approximation of the horizontal distance to the potential sources of fusaria. If we assume that the BVS (at a height $z_{BVS} = 1$ m AGL) and the UAV (at a height $z_{UAV} = 100$ m AGL) samplers are simultaneously sampling from a common source, then the ratio in concentration C_{UAV}/C_{BVS}, which would be sampled is approximately,

$$\frac{C_{UAV}}{C_{BVS}} = \exp\left(\frac{1}{2\sigma_z^2}\left[-(z_{UAV}-z_s)^2 + (z_{BVS}-z_s)^2\right]\right) \tag{12}$$

This equation can be used to estimate the distance, x, to the source using C_{UAV}/C_{BVS} and an assumed height for the source, z_s. In fact, only the relative height between the source and each sampler is important. Since z_s is unknown, we can assume it is close to ground level. Ground level near the Burkard sampler is within a few meters of the elevation of the New River and thus close to the minimum elevation for the surrounding 4 km radius. Considering elevation on the farm, the highest point within a 1 km horizontal radius from the Burkard site is located at 80 ± 4 m above the elevation of the Burkard sampler. This elevation is representative of the maximum elevation in the surrounding 4 km radius as well. Thus, we will consider two cases, $z_{BVS} - z_s = 1$ m ($z_{UAV} - z_s = 100$ m) and $z_{BVS} - z_s = -80$ m ($z_{UAV} - z_s = 19$ m), referred to as the min elevation and maximum elevation distances, respectively, in order to get an idea of the range of distances. We note that there is a singular case where the source is midway between the BVS and UAV, at a vertical displacement of $z_s^* = 1/2(z_{UAV} + z_{BVS}) = 50.5$ from each, where Eq. (12) cannot be used to estimate distance to the source. A limitation of the method is that if $C_{UAV}/C_{BVS} < 1$, we can only calculate a distance for $z_s < z_s^*$ and if $C_{UAV}/C_{BVS} > 1$, we can only calculate a distance for $z_s > z_s^*$. Thus, concurrent samples that provide a minimum elevation distance will not provide a maximum elevation distance, and vice versa. At the ratio of exactly 1, the distance to the source would be infinite, and close to 1, large distances are given. We only collect an integer number of spores, and given the high sensitivity of the distance estimate to the ratio C_{UAV}/C_{BVS} when it is close to 1, we exclude as unreliable those distance estimates for ratios C_{UAV}/C_{BVS} which are between 0.9 and 1.1 (these bounds are based on considering the sensitivity in the ratio due to the addition or subtraction of a single spore in either the UAV or BVS sample). Given current volumetric sampling rates, this sensitivity to ±1 s spore effectively renders our method unable to estimate distances beyond about 4 km. Furthermore, from our data, sensitivity to ±1 spore can change the distance estimate by an average of ±11%, but when low numbers of spores were present (<5), this sensitivity can be as high as ±40%.

2.6. Data analysis methods

JMP Pro 10 (SAS Institute Inc., Cary, NC) was used to conduct statistical analyses. Linear regression analyses were performed with sampling time intervals (0900–1100, 1100–1300, 1300–1500, 1500–1700), altitude (1 m and 100 m), and season (spring, summer, fall, and winter), with spore concentration as the response variable. ANOVA was used to analyze the differences among species and spore types that were associated with the calculations of UAV collection efficiencies. A correlation analysis was conducted to determine the association between BVS sampling interval and spore concentration to determine if increased sampling time was associated with an increase in spore collections.

3. Results

Collection efficiencies for UAV and BVS samplers are shown in Table 1. Results indicated that the collection efficiency of the BVS (99.97%) was much higher than the UAVs (1.34%), when considering macroconidia only (the only spore type shared among all of the species listed). UAV collection efficiencies were significantly different among different species ($P = 0.02$) and among spore types ($P < 0.01$) of *Fusarium*. These species and efficiencies are given in Table 1. The UAV sampling efficiencies ranged from 0.16% to 4.06%, with a mean ± standard error of 1.34% ± 0.39% (Table 1). The BVS sampling efficiencies were close to 100% (Table 1), with an unweighted average of 99.97%. Thus, we used $E_{eff}^{UAV} = 1.34\%$ and $E_{eff}^{BVS} = 100\%$.

A total of 2218 CFUs of *Fusarium* (615 CFUs from 104 BVS sampling periods, and 1603 CFUs from 180 UAV sampling periods) was collected over crop fields in Blacksburg, VA over four calendar years (2009–2012) (Table 3). Corrected spore concentrations ranged from 0 to 13.48 and 0 to 23.32 spores m^{-3} for the BVS and the UAVs, respectively. Multiple linear regression analyses indicated that there were significant differences in season ($P < 0.01$) and height ($P < 0.05$), but not time of sampling ($P = 0.47$). There were 30 BVS sampling intervals during which two UAV sampling flights were

conducted per interval. These 30 flight pairs, which had flight samples separated in time by 0.83 and 2.92 h (average 1.27 h and standard deviation 0.38 h), showed a high correlation ($r = 0.798$) in spore concentrations. This is consistent with earlier results which considered spore concentration correlations as a function of the time between sample flights (Lin et al., 2013).

Seasonal trends in spore concentrations are shown in Fig. 2. Spore concentrations were generally higher in the fall, spring, and summer, and lower in the winter (Fig. 2). For 104 BVS sampling periods, spore concentrations ranged from 0.11 to 2.93 spores m^{-3} across all seasons (35 in the spring, 16 in the summer, 44 in the fall, and 9 in the winter). For 180 UAV sampling periods, spore concentrations ranged from 0.31 to 1.56 spores m^{-3} for all seasons (57 in the spring, 44 in the summer, 59 in the fall, and 20 in the winter). The lowest mean spore concentrations for the BVS were observed in the winter, and the highest mean spore concentrations for the BVS were observed in the spring. Spore concentrations were generally higher with the BVS than those with UAVs for both seasonal and hourly data (Table 3, Figs. 2 and 3). There was no significant correlation between BVS sampling interval and spore concentration ($r = -0.04$, $P = 0.69$; Fig. 4).

For BVS sampling, there were 30 sampling periods in which no (zero) CFUs were recovered across all seasons (spring 9/35, summer 1/16, fall 14/44, and winter 6/9). For UAV flights, there were 18 sampling periods in which no (zero) CFUs were recovered across all seasons (spring 6/57, summer 2/44, fall 3/59, and winter 7/20). In spring, there were 30 simultaneous BVS-UAV sampling intervals, during which 1 or more CFU was collected by the UAV. Of those simultaneous sample intervals, 73% (22/30) included times during which 1 or more CFU was collected by the BVS, i.e., 22 had non-zero ratios C_{UAV}/C_{BVS}, while the remaining 8 have ratios C_{UAV}/C_{BVS} of infinity. The ratios for the other seasons are: summer 96% (22/23), fall 76% (31/41), and winter 22% (2/9). Only twice were zero CFUs collected with both the BVS and UAV. Technically, during those times for which we have a ratio C_{UAV}/C_{BVS} of infinity, we should assign infinite distance to source. In practice, we leave these out of the average distance calculation.

For those times when both BVS and UAV had non-zero collections, we formed the ratio C_{UAV}/C_{BVS}, and used a Gaussian plume transport model, Eq. (12), to estimate the average distance to the sampled source during the seasons (Fig. 5). The minimum elevation distances (i.e., assuming a source 1 m below the BVS sampler) are as follows: spring (1.5 ± 0.71 km; $n = 32$), summer (2.0 ± 0.90 km; $n = 23$), fall (1.3 ± 0.67 km; $n = 21$), and winter (4.1 ± 0.00 km; $n = 2$), where we give the mean $\pm$ standard deviation and n is the sample size of concurrent measurements meeting our criteria (there were only two measurements in winter showing identical concentration ratios). There were no concurrent measurements meeting our criteria for winter. Maximum elevation distances (i.e., assuming a source 81 m above the BVS sampler) are as follows: spring (1.2 ± 0.15 km; $n = 4$), summer (1.1 ± 0.36 km; $n = 11$), and fall (1.1 ± 0.35 km; $n = 7$). There were no concurrent measurements meeting our criteria for winter. Averaging over both the minimum and maximum elevation distances yields: spring (1.4 ± 0.67 km; $n = 36$), summer (1.7 ± 0.90 km, $n = 34$), fall (1.2 ± 0.61 km), and winter (4.1 ± 0.00 km; $n = 2$).

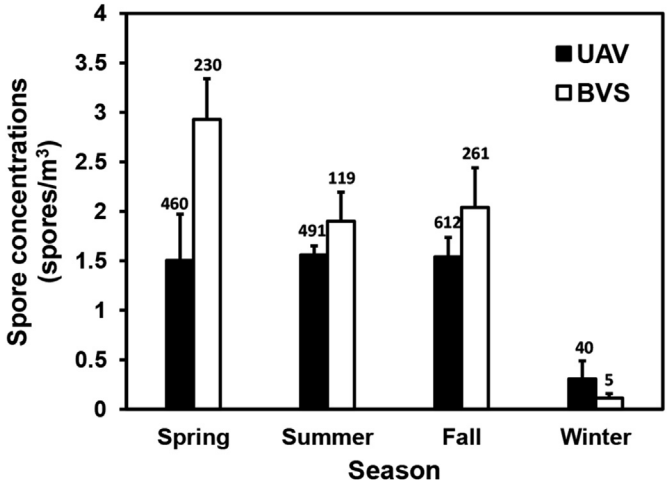

Fig. 2. Seasonal collections of *Fusarium* with a Burkard volumetric sampler (BVS) 1 m above ground level and an unmanned aerial vehicle (UAV) 100 m above ground level. Samples were collected at Virginia Tech's Kentland Farm in Blacksburg, VA over four calendar years (2009, 2010, 2011, & 2012). CFUs were converted to spore concentrations (number of viable spores m^{-3} of air sampled). Mean spore concentrations (spores m^{-3}) across seasons between UAV and BVS are reported. Error bars represent the standard error of the mean. The number of collections contributing to each mean is denoted above each bar.

4. Discussion

Though many fungi (and some oomycetes) of relevance to plants, domestic animals, and humans may be transported over long distances in the atmosphere, it is often difficult to pinpoint the source(s) of inoculum at both local (farm) and regional (state or country) scales. Here, we leveraged technologies with autonomous UAVs and a BVS to examine associations between assemblages of *Fusarium* collected 1 m and 100 m above the ground at a single sampling location in Blacksburg, VA, USA. A total of 2218 colony forming units (CFUs) of *Fusarium* (615 CFUs from 104 BVS sampling periods, and 1603 CFUs from 180 UAV sampling periods) was collected over four calendar years (2009–2012). This study extends the work of others that have examined spore concentrations of different biological agents at different heights in the atmosphere (e.g., Aylor, 1998; Dasgupta, 1988; De Jong et al., 2002; Khattab and Levetin, 2008). To our knowledge, this is the first study to link simultaneous observations of fungi at 1 m and 100 m above ground level. Such knowledge could help identify environmental signatures of air pollution and climate change (Frenguelli, 2013), and

Table 3
Colony forming units (CFUs) of *Fusarium* collected with a Burkard volumetric sampler (BVS) 1 m above ground level and an unmanned aerial vehicle (UAV) 100 m above ground level. Samples were collected at Virginia Tech's Kentland Farm in Blacksburg, VA over four calendar years (2009, 2010, 2011, & 2012). CFUs were converted to spore concentrations (number of viable spores m^{-3} of air sampled).

Year	Number of UAV sampling periods	CFUs from UAVs	Number of BVS sampling periods	CFUs from BVS	Mean spore concentrations from UAVs (spores m^{-3})[a]	Mean spore concentrations from BVS (spores m^{-3})[a]
2009	14	70	10	87	0.52	1.81
2010	24	441	18	163	1.28	2.56
2011	116	1028	62	354	1.72	2.43
2012	26	64	14	11	0.56	0.27

[a] Spore concentrations from UAVs and a BVS were calculated using Eq. (10) in the text.

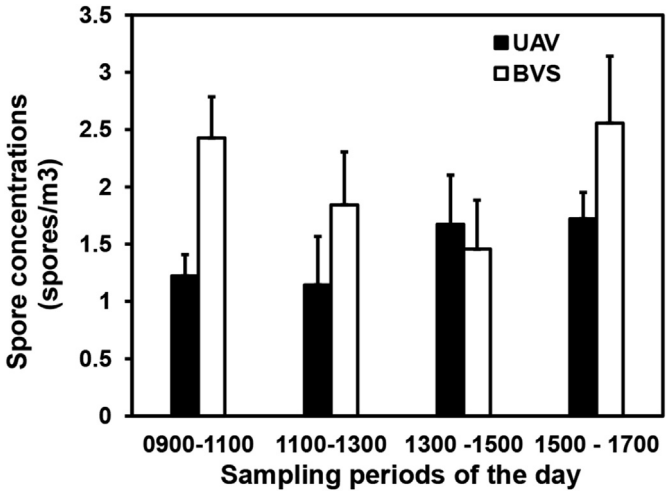

Fig. 3. Mean spore concentrations (number of viable spores m^{-3} of air sampled) of *Fusarium* collected with a Burkard volumetric sampler (BVS) 1 m above ground level and an unmanned aerial vehicle (UAV) 100 m above ground level across four different sampling intervals (0900–1100, 1100–1300, 1300–1500, and 1500–1700). Error bars represent the standard error of the mean. Samples were collected at Virginia Tech's Kentland Farm in Blacksburg, VA over four calendar years (2009, 2010, 2011, & 2012).

assist in bridging the gap between local (farm) (Aylor et al., 2011) and regional (across states or continents) transport of microorganisms (Smith et al., 2011a, 2011b).

Many factors need to be considered when trying to determine how spore collections vary with height (e.g., 1 m vs. 100 m AGL), including changes in local source concentrations of *Fusarium*, variations due to weather patterns, and the biophysical processes that govern spore transport (Aylor, 1999, 2003; Isard and Gage, 2001). Here, the number of spores collected 1 m and 100 m AGL were converted to spore concentrations (spores m^{-3}) by dividing the spore count by the amount of air sampled in cubic meters and the collection efficiency (related to inertial effects of spores relative to the air). The mean collection efficiency of the BVS (99.97%) was much higher than the UAVs (1.34%) when considering different sizes of macroconidia. Sampling efficiencies for the UAVs varied

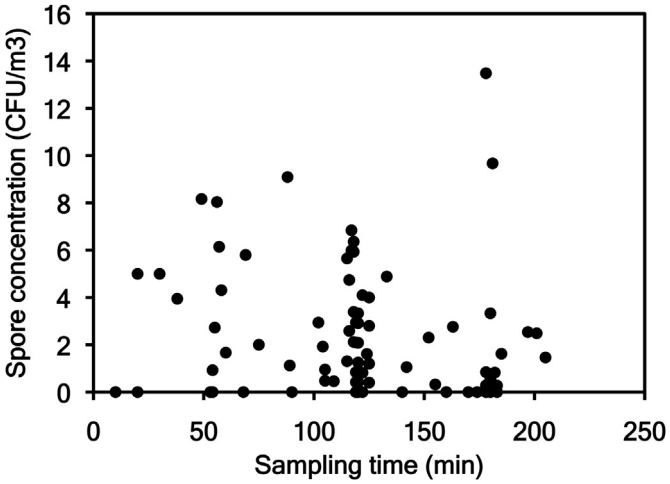

Fig. 4. Spore concentrations (CFU/m^3) of *Fusarium* collected from a Burkard volumetric sampler (BVS) across 104 different sampling intervals ranging from 10 to 205 min. There was no significant correlation between BVS sampling interval and spore concentration ($r = -0.04$, $P = 0.69$).

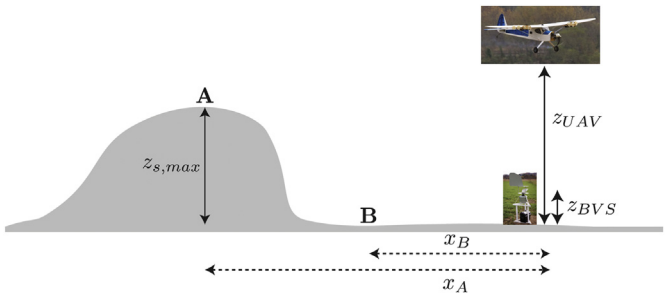

Fig. 5. Schematic of distance to source estimation. From Eq. (12), the horizontal distance from the sampling location to the unknown source can be estimated assuming an elevation of the source. To put bounds on the horizontal distance, we consider both a maximum elevation (e.g., A) and minimum elevation (e.g., B), based on the extremes of the topography within a several km radius of our sampling location. This gives two horizontal distance estimates.

with spore size, which is due in part to differences in spore shape (Aylor, 1993; Aylor et al., 2006; Leslie and Summerell, 2006). Since collection efficiency is a function of the settling velocity and spore size, collection efficiencies depend on the species of *Fusarium* being sampled, with each species expected to have a unique collection efficiency (Aylor, 1993; Aylor et al., 2006). This, however, is also complicated by the fact that one species of *Fusarium* can produce as many as four different spore types that have the potential to become airborne (macroconidia, microconidia, mesoconidia, and ascospores) (Leslie and Summerell, 2006). Unfortunately, our spore collection method (recovery of CFUs of *Fusarium* on agar plates) did not allow us to differentiate the type of spore that was collected. Knowledge of the fraction of each spore type being collected for each species of *Fusarium*, if known, could be used to refine our estimates of spore concentrations.

Spore concentrations ranged from 0 to 13.48 and 0 to 23.32 spores m^{-3} for the BVS and the UAVs, respectively. Spore concentrations were generally higher in the fall, spring, and summer, and lower in the winter. Seasonal climate changes, such as temperature, rainfall, humidity, ultraviolet (UV) light, and wind, influence spore dispersal (Dill-Macky and Jones, 2000; Jones and Harrison, 2004; Lyon et al., 1984). Overall, we observed dramatic decreases in CFUs (95% for BVS and 78% for the UAVs) in the winter. Environmental conditions in Blacksburg, VA are considered to be unfavorable for spore production in the winter, and might help explain the decreased number of spores observed in the winter (at least for those spores coming from local sources). Consequently, we speculate that spores collected with the UAVs during the winter were likely originating from more distant sources, perhaps in warmer regions.

Spore concentrations from the BVS were generally higher than those from the UAVs for both seasonal and hourly collections on average (though the UAV concentration had a higher maximum). This is consistent with previous reports demonstrating a general decrease in spore concentrations with increasing height from ground level (Bergamini et al., 2004; Chakraborty et al., 2001; Hirst and Stedman, 1967; Khattab and Levetin, 2008). Though the actual contribution of local and more distant sources to atmospheric assemblages of *Fusarium* remains unclear, collections at 1 m were significantly greater than those at 100 m.

The estimated average transport distance to a ground-level source (1 m below the BVS sampler) was 1.5 km for the spring, 2.0 km for the summer, 1.3 km for the fall, and 4.1 km for the winter; all values lying within one standard deviation of the others. Summer had the highest variability (±0.90 km), followed by spring and fall (±0.71 km and ±0.67 km, respectively). Spring, summer, and fall provided both minimum and maximum elevation distance

estimates, with the maximum elevation distances all being smaller than the minimum elevation distances, but with values lying within one standard deviation. Winter provided the fewest simultaneous samples meeting criteria for distance estimates. However, winter was the season with an anomalously high percentage of simultaneous sampling periods (55%), during which at least one CFU was collected with the UAVs but no CFUs were collected with the BVS. This is consistent with a distant (for instance, >4 km) source. Thus, samples collected during the winter were likely coming from more distant sources, compared to samples from the other seasons.

Future work aims to identify environmental signatures that could predict atmospheric loads of *Fusarium*, which ultimately could inform measures of air pollution and even climate change (BozorgMagham et al., 2013; Isard et al., 2005; Strange and Scott, 2005; Tallapragada et al., 2011). Such work could leverage the identification of each *Fusarium* colony to the species level, and the associations of each species with the potential for disease and mycotoxin production (Schmale et al., 2012).

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