Detection of airborne *Coccidioides* spores using lightweight portable air samplers affixed to uncrewed aircraft systems in California's Central Valley

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ABSTRACT

Coccidioidomycosis is an emerging fungal infection caused by inhalation of *Coccidioides* spp. spores. While airborne dispersal is critical to *Coccidioides* transmission, limited recovery of the pathogen from air has hindered understanding of the aerosolization and transport of spores. Here, we examine uncrewed aircraft systems (UAS) with portable, active air samplers as a novel means of capturing aerosolized *Coccidioides* and characterizing emissions and exposure risk. We sampled in September 2023 in eastern San Luis Obispo County, California, in an area with confirmed *Coccidioides immitis* in soils. We completed 41 20-minute flights across 14 sites using UAS equipped with an 8 L/min bioaerosol sampler and a low-cost particulate matter sensor. We sampled source soils and air under ambient conditions using one UAS at 1-10 m altitude, and under a simulated high-dust event using two UAS, one at <2 m altitude and one at 5-12 m. We detected *Coccidioides* DNA in two of 41 air samples (4.9%), both under ambient conditions at 8 m altitude, representing the highest known altitude of airborne *Coccidioides* detection. Spatially explicit UAS-based sampling could enhance understanding of *Coccidioides* aerobiology and enable detection in hard-to-reach or hazardous air masses, including dust storms and wildland fire smoke.

INTRODUCTION

Coccidioidomycosis, commonly known as Valley fever, is an emerging fungal infection caused by the inhalation of aerosolized spores of the *Coccidioides* genus. Infection primarily causes respiratory symptoms including cough, fever, fatigue, and shortness of breath, but in 1-5% of cases, the disease can progress to a chronic or severe disseminated state (1). The fungus is predominantly found in soils of desert and semi-arid regions of the southwestern United States, particularly Arizona and California, where *C. posadasii* and *C. immitis,* respectively, occur (1,2). Since 2000, coccidioidomycosis incidence has increased nine-fold in California, most rapidly in geographic areas outside the traditionally considered endemic zones (3,4). This emergence appears linked to hydroclimate fluctuations and warming that enable *Coccidioides'* growth, aerosolization, and dispersal (4–6). Research finding positive associations between fine mineral dust exposure and coccidioidomycosis incidence (7,8), coupled with outbreaks of coccidioidomycosis among outdoor workers exposed to dust via construction and wildfire fuel hazard reduction and fireline construction (9,10) suggest that soil disturbance by both natural and anthropogenic mechanisms may aerosolize fungal spores (2). However, there are gaps in our understanding of the conditions facilitating aerosolization, airborne concentration, and transport of *Coccidioides* under both ambient air conditions and during specific events that elevate particulate matter and dust concentrations, such as dust storms and wildfires.

Understanding how, where, and when fungal spores are aerosolized and transported is crucial for developing strategies to predict and reduce exposure to *Coccidioides*, such as the development of near-term forecasting systems for elevated exposure risk. Even as reliable methods for detecting *Coccidioides* in soil are well established (11), detection of airborne fungal spores is technically challenging (12–15). Most studies involving sampling for airborne *Coccidioides* have relied on active air filtration using fixed-location high-volume devices that intake 100-425 L/min of air for up to 24 hours (12,13,16,17), including large-scale surveillance efforts in endemic regions of Arizona (13,16). While such studies have enabled valuable preliminary understanding of airborne concentrations of *Coccidioides*, there are substantial obstacles to achieving scalable

stationary, high-volume sampling networks due to technology gaps as well as implementation costs and feasibility (18). Meanwhile, stationary low-volume filtration and passive sampling techniques that collect settling dust (and, potentially, spores) have been limited in their successful recovery and detection of *Coccidioides* DNA (12,14,15,19). Thus, the processes facilitating aerosolization, the airborne concentrations of *Coccidioides* spp. and their transport remain poorly understood, underscoring the need for new approaches capable of capturing and recovering airborne spores.

Small and lightweight portable samplers show promise for active collection of bioaerosols– either air or ground-based – using air flow rates more relevant to human exposure risk (20,21). Attachment of portable samplers to the fuselage of uncrewed aircraft systems (UAS) further adds flexibility to where air can be sampled, and has been used to assess the composition of microbes in smoke to understand how wildland fires mobilize organic and inorganic particles including dust and bioaerosols (20,22,23). However, it is currently unknown whether similar methodologies might enable detection of airborne *Coccidioides*. In this study, we assessed the feasibility of UAS equipped with lightweight, active aerosol samplers in recovering *C. immitis* from the air—a sampling approach that may expand opportunities for characterizing the aerosolization and transport of fungal spores and pathogens to and from environmental reservoirs (23).

MATERIALS AND METHODS

Site description

We conducted our study in September 2023 in the Carrizo Plain National Monument in San Luis Obispo County, California, USA (Figure 1), a preserved arid grassland ecosystem that provides habitat for burrowing mammal species, including the endangered *Dipodomys ingens* (giant kangaroo rat) and *Vulpes macrotis mutica* (San Joaquin kit fox), known to be susceptible to *Coccidioides* infection (24,25). The Carrizo Plain is highly endemic for *Coccidioides immitis*, as previous work has isolated *C. immitis* DNA from 26% of small mammal burrow soils within the monument (26).

Air sampling

We completed 41 flights across 14 sites using two DJI Matrice 300RTK (SZ DJI Technology Co., Ltd.) quadcopter UAS with 1.5 kg payloads, following methods described by Kobziar and colleagues (20,22). At each site, we implemented two sampling schemes designed to compare ambient with wind-affected air masses (Figure 2A). First, we sampled air under ambient conditions (hereafter, "solo ambient") by conducting solo UAS flights at 1.2 to 10.5 m altitude. Next, we sampled air using two UAS under a paired sampling scheme designed to simulate high-dust conditions. We flew one UAS below five m altitude to mobilize dust via air turbulence from rotor downwash ("paired low altitude"), and concurrently flew a second drone 5-20 m downwind at 5-10 m altitude ("paired high altitude") to capture aerosolized dust potentially containing spores. Flight records were downloaded and de-encrypted using AirData software (27). The UAS altitude, or height above ground level (HAGL), was determined using their downward-facing infrared sensors for altitudes less than 10 m, and the barometric altitude paired with a 10 m spatial resolution digital elevation model (DEM) for altitudes over 10 m.

Each UAS was equipped with a battery-powered compensating Leland Legacy sampling pump and button aerosol sampler (SKC Inc., Eighty Four, PA, USA) (22,28) that drew in air at a volumetric rate of 8 liters per minute (L/min), split and filtered through two 2 µm PTFE (Teflon®)

filters. Flights sampled 160-216 L of air over 20 to 27 minutes. A delay in air sampling was programmed to ensure that air was sampled only after the UAS reached the desired flight altitude. After each flight, both PTFE filters were brought into a portable, sterile glovebox, and extracted from the button sampler and pooled together in a 50-mL centrifuge tube before being transferred to coolers and stored on ice. The impactor was cleaned with 70% ethanol between filter loading to minimize cross-sample contamination. Samples were transferred daily to -20°C and maintained at this temperature until transferred to -80°C within four days. To help account for possible contamination, two to three procedural blanks were collected daily in the field. Briefly, new filters were installed in the clean button sampler and allowed to sit for 1 minute, then removed into 50-mL centrifuge tubes in a sterilized glovebox using the same procedures as for positive samples. Blanks and samples were stored and analyzed identically.

Measurement of environmental covariates

During flight, the UAS recorded continuous measures of speed, latitude and longitude, and height above ground level. Each UAS had an attached PurpleAir PA-II-SD (PurpleAir, Inc.), which included two PMS5003 particulate matter optical sensors (Plantower, Beijing, China) (22,28). The PurpleAir were programmed to record data every eight seconds on air temperature, relative humidity, dewpoint, and particulate matter smaller than 1.0, 2.5, and 10 microns ($PM_{1.0}$, $PM_{2.5}$, and $PM_{10.0}$). Technical issues prevented one PurpleAir monitor from recording data during eight of the 41 flights. All UAS data manipulation and analysis was done in Python (version 3.10) and R (version 4.3.2). We acquired hourly data on average and maximum wind speed from the Remote Automatic Weather Stations (RAWS) US Climate Archive, which has a station located within the Carrizo Plain (29). These observations were linked to the UAS flights based on closest match to time of flight.

Soil sampling

To examine whether *C. immitis* in air was associated with *C. immitis* in soils, we collected soil samples (12 per site, total n=168) at locations beneath the UAS airspace of each flight site. We prioritized sampling from inside rodent burrow entrances, where prior work has found a high probability of detecting *C. immitis* (26,30,31) and from exposed surface soil nearby burrows, as these soils may be more readily dispersed into the air than those collected from inside burrows. Per site, six samples were collected from inside burrow entrances and six from nearby surface soil. Our sampling design followed a combined radial and interrupted belt transect approach (Figure 2B) adapted from prior work (32). Herein, six 50-m radii were measured from a centroid at 60-degree angles. Along each transect, soil from the top one cm of the surface was collected at approximately 10 m, 30 m, and 50 m and pooled into one tube (total volume 25-35 mL). We prioritized sampling of loose soils that could be readily dispersed and appeared to have been excavated from burrows by rodent activity. We also sampled one rodent precinct (i.e., cluster of burrows) along each transect, pooling samples from up to three burrow entrances per precinct (total volume 10-30 mL) and giving priority to precincts that appeared active via presence of feet and tail markings, clipped vegetation, and fecal pellets. Soils were collected using a stainless-steel scoop with a 10-inch handle, placed into sterile, 50-mL centrifuge tubes, and sealed in zip-top plastic bags. Stainless-steel scoops were thoroughly cleaned with 70% ethanol between samples to prevent contamination. Samples were stored at ambient conditions until analysis, following standard protocols for the analysis of *Coccidioides* in soils (32–34). In the laboratory, equal volumes of soil (250 μL) were subsampled from each 50-mL tube for DNA extraction.

DNA extraction and detection of *Coccidioides DNA*

Genomic DNA was extracted from soil samples using the DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany), and from filters using a customized phenol-chloroform extraction procedure (35) (personal correspondence: Bridget Barker and Daniel Kollath). The two 2 µm PTFE (Teflon®) filters collected from each flight were pooled for extraction and analysis. A laboratory blank was processed alongside the samples and field blanks. Centrifuge tubes containing the filters were washed with lysis buffer to maximize DNA yield. DNA was quantified using a Qubit™ fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, USA). As in prior work, samples were diluted to 12 ng/µL and assessed for presence of *Coccidioides* DNA using the CocciEnv qPCR assay (11). Samples were run in quadruplicate and classified as positive for *Coccidioides* if at least three of four replicates had a cycle threshold (Ct) value below 40, following prior work (14).

Statistical analyses

We mapped detailed flight paths and summarized flight characteristics, environmental data, and sample positivity rates across sites. We compared the prevalence of *C. immitis* across sample types (solo ambient, paired low altitude, paired high altitude, surface soil, and burrow soil) and examined the relationships between environmental factors (PM concentration, relative humidity, temperature, height above ground, time of day, and wind speeds), flight characteristics (altitude, flight duration, distance travelled, and volume of air sampled) and detection of *C. immitis* in air samples using univariate logistic regression. Missing data resulting from the UAS PurpleAir monitor operating error were removed from analysis. The US Environmental Protection Agency's correction equations were used to estimate $PM_{2.5}$ concentrations from PurpleAir sensor particulate data (36). Statistical analysis was performed in R (version 4.3.2) (37).

RESULTS

Out of 41 air samples collected between September 22-24, 2023, two were positive for *C. immitis* DNA (4.9%) (Table 1). These positive samples were collected under ambient conditions (i.e., ambient solo flights) at mean heights of 7.9 m and 8.4 m above ground, at two different sites (Figure 1). Meanwhile, out of 168 soil samples collected, 45 were positive for *Coccidioides* DNA (27%). The positivity rate of soil from inside burrows was 32.1%, and from surface soil, 21.4% (Table 1). The proportion of positive soil samples at any given site ranged from 0-66% (Figure 1). At both sites from which positive air samples were collected, *C. immitis* DNA was detected in the soil beneath the UAS airspace. All laboratory and field blanks collected had no to negligible detectable DNA quantities (<0.07 ng/uL) and all were negative for *Coccidioides* DNA (see Methods).

We found no significant associations between *Coccidioides* detection in airborne samples and environmental data recorded during flights or flight characteristics. Mean flight duration was 22.4 minutes (range: 20-27 minutes), corresponding to 160-216 L of filtered air. The mean height above ground for ambient flights was 6.3 m (range: 1.2-10.5 m). For the paired sampling scheme, the mean height above ground for low flights was 1.08 (range: 0.84-1.44 m) and for high flights was 7.53 (range: 5.21-11.77 m). The mean concentrations of $PM_{1.0}$, $PM_{2.5}$, and $PM_{10.0}$ were 21.8 µg/m³ (SD 4.4), 21.3 µg/m³ (SD 3.0), and 40.0 µg/m³ (SD 7.1), respectively. There were no significant differences in mean $PM_{1.0}$, $PM_{2.5}$, or $PM_{10.0}$ concentration based on flight type for either drone (Supplemental Table 1). Using data gathered from the Carrizo RAWS weather station, the

average hourly temperature during sampling was 25.3°C, with 31.4% relative humidity, and average wind speeds of 7.3 mph.

DISCUSSION

This is the first study, to our knowledge, to detect *C. immitis* in the air using portable active air samplers attached to UAS. We recovered *C. immitis* DNA from two of 41 air samples collected by a UAS-based sampler, both of which were collected under ambient conditions at heights of approximately 8 m above ground. This represents the highest altitude where airborne detection of *Coccidioides* has been successful, as prior surveillance efforts have sampled air using fixed devices stationed at 1.4 m and 4.6 m above the ground (13,16). This study establishes UAS as a feasible sampling method for airborne *Coccidioides*, laying groundwork for future investigations to evaluate the atmospheric and environmental factors influencing emissions and transport of spores.

Contrary to our expectations, *C. immitis* was only detected during ambient flights and not during lower-altitude flights that were conducted specifically to disturb and aerosolize soils presumably containing spores. However, since we did not observe any difference in PM concentrations between ambient and lower-altitude flights, it is possible that the relevant size fraction of dust with potential to contain spores was not aerosolized during paired flights. Prior air sampling work in Arizona found no association between *C. posadasii* presence on air filters and dust storms (16). We found no association between soil presence and airborne presence of *Coccidioides*, though this may be due to limited power in having only two positive air samples. Given the potential for long-distance dispersal of fungal spores in the air (38) and evidence for an air mycobiome with distinct composition from the soil mycobiome under ambient conditions (19), it is possible that the spores we detected were dispersed via wind from farther distances.

We propose that use of UAS in air sampling can help understand environmental factors affecting *Coccidioides* dispersal in endemic regions, given its spatial precision via integrated GPS tracking functionality; ability to sample at varying altitudes and hard-to-reach locations; and ability to concurrently collect data from monitors affixed to the UAS (22). One compelling application of this technology could be to assess the role of fires – both wildfires and prescribed burns – in dispersing fungal spores and other bioaerosols, a key knowledge gap that has only begun to be addressed (23,39). In the process of burning organic soils and plant material, fires may directly emit as well as draw fungal matter, spores, and conidia into the smoke column via convective winds. The bioaerosols then have the potential to travel hundreds of miles on wind currents before being deposited or inhaled downwind of the fire (20,40–42). UAS have been employed to sample within wildland fire plumes at various specific altitudes, while allowing the operator to remain at a safe distance away (28,43). Further, past work has demonstrated the efficacy of utilizing UAS to sample viable microbiological aerosols within smoke plumes (20,22), finding that plumes contain higher concentrations and a greater diversity of microorganisms than ambient air (22). In epidemiologic studies, multiple outbreaks of coccidioidomycosis have been documented among wildland firefighter crews (9,44), Mulliken et al. (2023) documented a 20% increase in Valley fever (coccidioidomycosis) hospital admissions following wildfire smoke exposure in California (45), and Phillips et al. (2023) found increases in coccidioidomycosis incidence among residents in the two years following fires, for three large fires located near endemic population centers (46). Employing UAS to quantify the potential concentrations of aerosolized *Coccidioides* during wildfires would enable practical linkages with smoke models parameterized for pathogenic bioaerosol transport (47).

Our findings also suggest that portable button aerosol samplers sampling at lower flow rates (and even without UAS integration) could be leveraged to assess personal exposure to *Coccidioides* spores. We demonstrate isolation of *C. immitis* in only 176-184 L of air using lightweight portable samplers that filter air at a rate akin to human breathing (8 L/min). Our study found *C. immitis* DNA in 4.9% of air samples, similar to the positivity rate found in Phoenix, AZ (7.4%), which relied on high-volume fixed-location samplers that filtered 2,400 of liters of air (16).

While we demonstrated a novel approach for detecting airborne *Coccidioides*, our small positive sample size (i.e., 2 flights) precluded us from detecting associations between *Coccidioides* presence and environmental factors. Investigating these associations, as well as the relationships between *Coccidioides* presence in air and various land use types, dust-generating activities such as wildfires, and presence in the soil are compelling areas of future research. Given the public health threat of the emergence of coccidioidomycosis in the western United States, it is imperative to continue investigating how environmental and anthropogenic disturbances may facilitate the transport of *Coccidioides* spores and influence exposure risk.

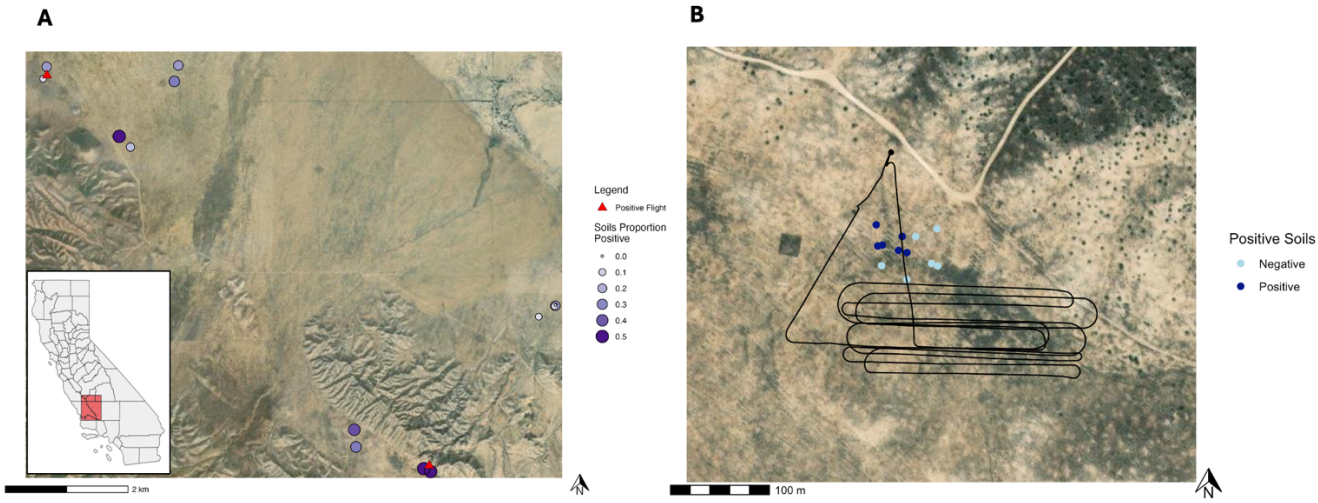

Figure 1: Maps of sampling locations in the Carrizo Plain, CA. (A) Layout of all sites sampled, where circles denote the proportion of positive soil samples at each site and red triangles indicate the two sites with positive flights. The inset highlights the approximate location of the Carrizo Plain National Monument in California. (B) The UAS flight path for site 14 is designated in a black line. Circles denote where soil samples were concurrently collected and whether each sample was negative (light blue) or positive (dark blue) for *Coccidioides*.

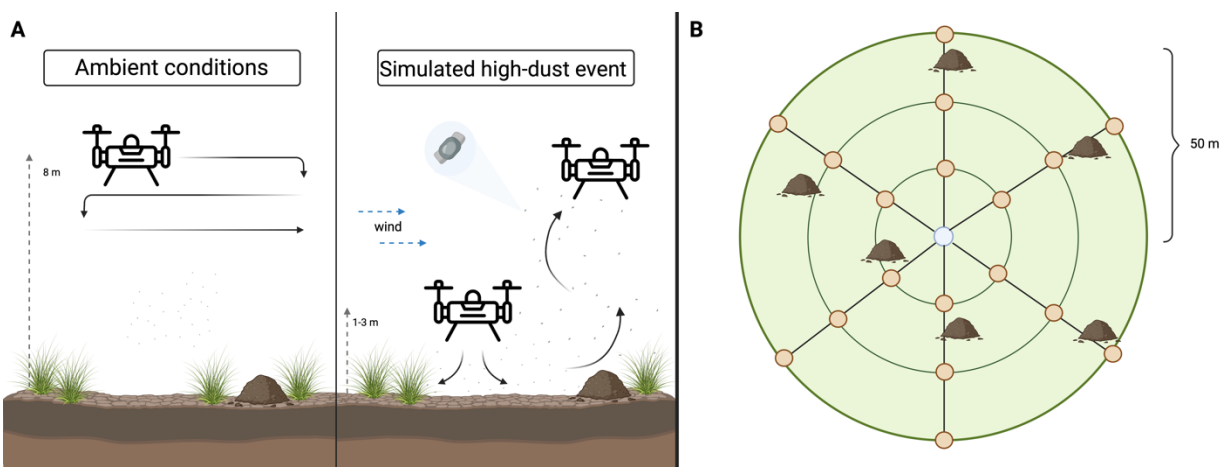

Figure 2: Schematics for air and soil sampling designs (A) Air sampling design for ambient and simulated high-dust flight types. (B) Systematic radial and interrupted belt transect soil sampling design.

Table 1: Positivity rate of air and soil samples collected in southeastern San Luis Obispo County, California, September 2023.

	***C. immitis* detected, n (%)**	**Overall, n**
Air samples	**2 (4.9)**	**41**
Ambient conditions	2 (13.3)	15
Paired high-dust conditions	0 (0)	26
Soil samples	**45 (26.7)**	**168**
Precinct (burrow) soil	27 (32.1)	84
Loose surface soil	18 (21.4)	84

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