

Testbeds for microbial source tracking using microfluidic paper-based analytical devices



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Summary

The project team has developed a molecular method to detect fecal contamination around animal operations using *Bacteroidales* DNA as a biomarker. The team is integrating this molecular method with data collected from weather stations to provide a risk heatmap for fresh produce operations in proximity with animal operations. Two different weather stations were compared using a testbed around animal operations over a 7-day period. The team also conducted preliminary evaluation of 30 collection flags at a stone fruit operation to determine the suitability of the flags in measuring *Bacteroidales* levels. We demonstrate that both these preliminary experiments point towards feasibility and help us select the design of weather stations (combination of the two setups used) and collection flags (taller poles with small area).

Objectives

1. Characterize testbeds for distribution of aerosolized fecal contamination and generate a risk heatmap that incorporates bioaerosol distribution and environmental metadata.
 - Screen and select weather station setup using an animal research operation in Indiana as a testbed (Testbed 1)
 - Determine the design of collection flags that is appropriate for stone fruit operations (Testbed 2)
2. Test the ability of microfluidic paper-based analytical devices (μPADs) to rapidly characterize bioaerosol distribution.
 - Simplify the image analysis procedure for obtaining data from μPADs

Methods

To record temperature, pressure, humidity, daily rain, UV and solar radiation, wind speed and direction along with air quality data, we tested two weather station models for comparison at a swine operation in Indiana (Testbed 1). We also generated preliminary heat maps of quantitative polymerase chain reaction (qPCR) data for *Bacteroidales* at a stone fruit operation (Testbed 2). The collection flags were placed at 100 m intervals from east to west and 70 m intervals from north to south in five blocks and collected after 7 days. The research team swabbed each flag, resuspended it in nuclease-free water and used it for qPCR assay. We detected *Bacteroidales* in all 60 flags and generated qPCR heat maps depending on the levels of *Bacteroidales*.

Results to Date

Data comparison of two weather station models by a paired t-test showed no significant differences (**Table 1**) and the patterns recorded for a period of 7 days were similar (Testbed 1) (**Figure 1**). The heat maps showed higher levels of *Bacteroidales* (Testbed 2, **Figure 2A**) in the northeast direction, possibly due to wind direction (**Figure 2B** and **C**). The first row with collection spots A1, A2, B1, B2, C1, and C2 had higher copy number of *Bacteroidales*, likely due to their proximity to the cattle farm. The results did not demonstrate a significant difference between small and large flags. The levels of *Bacteroidales* in these stone fruit operations (up to 10³ copies/cm²) are higher than what we have seen in the past with background levels for leafy green operations (~0–2 copies/cm²).

Benefits to the Industry

This study develops and tests a method to quantify the risk of fecal contamination in fresh produce operations that are in proximity to animal operations. We already see that there are differences across industries (e.g., leafy greens vs. stone fruit) and thus, suggest that a site-specific risk evaluation is beneficial. We are currently working on enabling this risk evaluation in the field using simple devices.

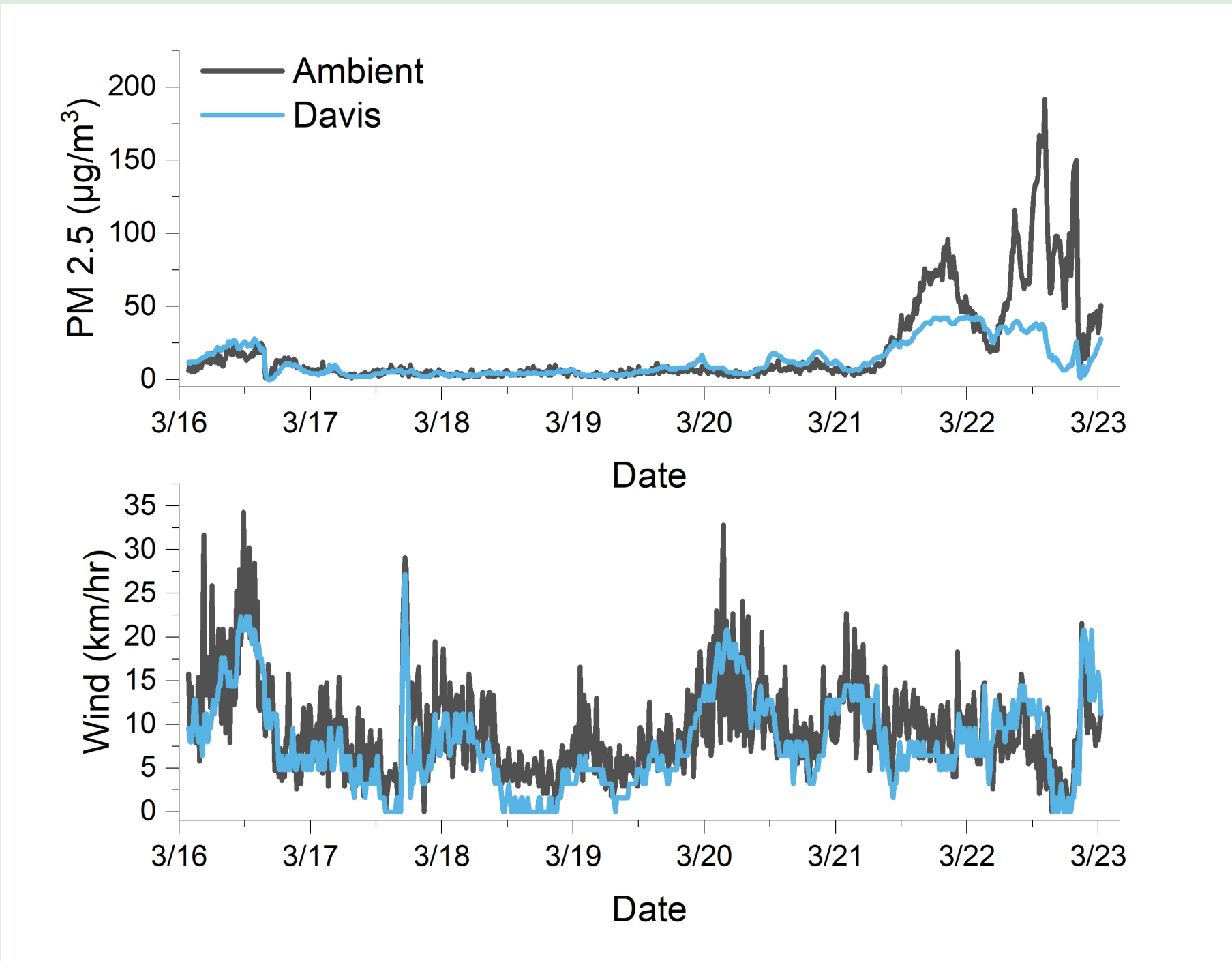


Figure 1. Data obtained from two different weather stations to compare the particulate matter ≤2.5 microns (PM2.5) (top) and wind speed (bottom) measurements.

Environmental parameters	Normality test (p-values)
Temperature (°C)	0.209
Humidity (%)	0.086
Pressure (Hg)	0.328
Wind (km/h)	0.555
High wind (km/h)	0.819
Solar radiation (W/m²)	0.096
UV Index	0.718
Dew point (°C)	0.674
PM 2.5 (µg/m³)	0.753

Table 1. Statistical difference by t-test in recorded parameters between two groups (i) Ambient WS-5000 and (ii) Davis Vantage Pro2 Plus. All data were recorded at 15-min time intervals and the daily data was averaged. Normality test was run using Sigma Plot 14.0. *P*-values higher than 0.05 shows that there was no significant difference between groups.

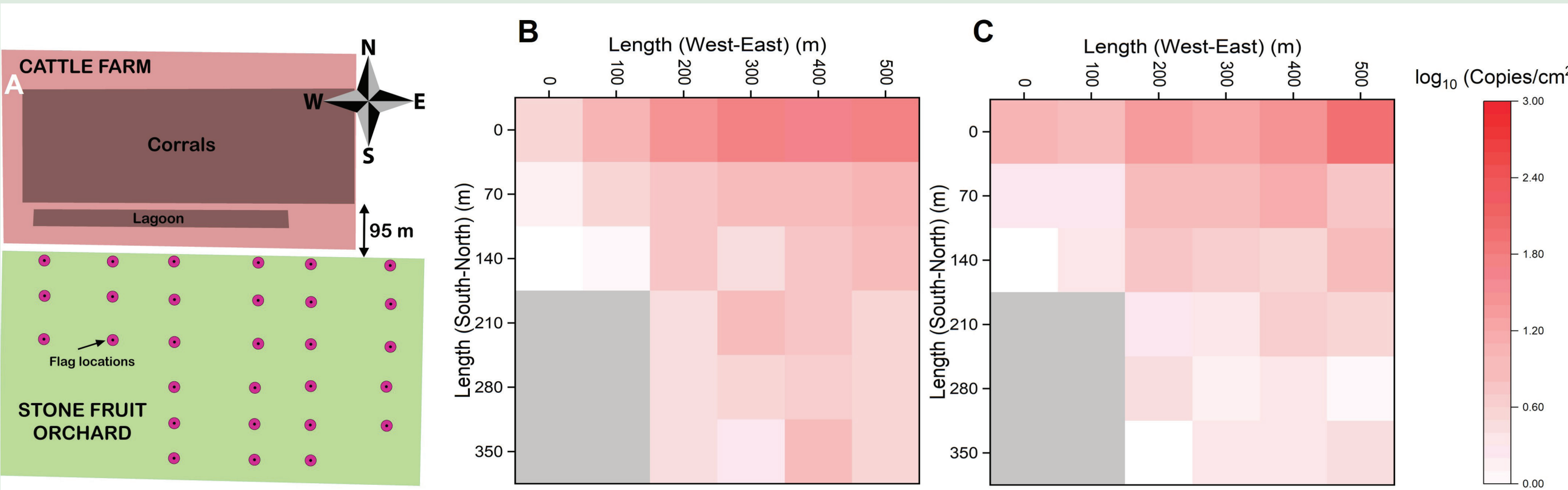


Figure 2. Preliminary qPCR analysis of collection flags in the stone fruit farm. A) Sites for the installation of flags. Points in panel represent the locations where flags were located. B) qPCR heat maps from small flag and C) large flag samples.