Project Title: Integrated management of tar spot of corn

Personnel

Principal Investigator	Dr. Yuan Zeng Assistant Professor of Plant Pathology Southern Piedmont AREC Virginia Tech	**Address:** 2375 Darvills Rd. Blackstone, VA 23824 **Email:** yuanzeng@vt.edu **Cell:** (970)372-8935

Objectives

1. Evaluate fungicide efficacy and timing (VT/R1 v.s. R3) on tar spot of corn.

- **Highlight**: this objective was partially completed (*i.e.,* VT/R1) but successful. The scheduled fungicide application at the R3 growth stage was missed due to management oversight.
- The graduate student funded by this project attended the annual Plant Health meeting to present findings on fungicide efficacy against common foliar corn diseases and pathogen monitoring in corn fields.

2. Conduct a greenhouse trial to evaluate corn hybrid susceptibility.

- **Highlight**: We evaluated the susceptibility of two hybrids to tar spot disease using a growth chamber assay as it provided a better-controlled environment compared to a greenhouse. The objective was completed and successful, and both hybrids were confirmed to be susceptible to tar spot disease under growth chamber conditions.

3. Build a fungicide application decision support tool for improved management of tar spot in Virginia.

- **Highlight**: Spore trap rods were collected from all three 2024 field locations at a bi-weekly schedule from planting to harvest, and a subset of metagenomic sequencing data of spore DNA was analyzed. The relationship of weather variables with tar spot disease severity was analyzed. The student also worked on developing scripts for tar spot disease forecasting models using different analytical approaches.

Results Achieved

Objective 1: We conducted uniform field trials in the 2024 growing season at the following three locations: New Market 1 (Shenandoah County; was reported as a high tar spot disease pressure location), New Market 2 (Shenandoah County; was reported as a low tar spot disease pressure location), and Blacksburg (Montgomery County; tar spot was not reported in previous years). Two corn hybrids and seven chemical treatments (Table 1) were evaluated in each field trial using split-plot designs, with main plots assigned to each hybrid and subplots assigned to chemical treatment arranged in randomized complete block designs with three blocks. Fungicide applications were applied according to labeled rates at the standard corn tassel or silk growth stage (VT/R1). Disease severity ratings were conducted within the center two rows at a 14-day interval from fungicide application till plants had senesced.

Table 1. Hybrid and fungicide information.

Hybrids	Fungicide and rate	FRAC group
P1380Q (advertised as a tolerant hybrid)	Adastrio; 7 fl oz/ac	3+7+11
	Trivapro; 13.7 fl oz/ac	3+7+11
	Veltyma 3.34 SC; 7 fl oz/ac	3+11
	Fortix; 4 fl oz/ac	3+11
	Lucento; 5 fl oz/ac	3+7
P1903AM™ (advertised as a susceptible hybrid)	Xyway; 15.2 fl oz/ac	3
	Non-treated control	-

- Tar spot was found in the New Market 1 trial (*i.e.,* irrigated field) but not in the New Market 2 (*i.e.*, non-irrigated field) or Blacksburg trials. The disease appeared late in the season when plants were approximately at the R4 stage this year, and its severity was relatively low (0-6%; Fig. 1) but higher than what was reported in 2023 (0-2%).
- No statistical differences in tar spot disease severity in the New Market 1 location were found between non-treated control and all six fungicides applied at the VT/R1 stage. However, tar spot disease severity in the non-treated control, as well as in plots treated with Adastrio (3+7+11), Trivapro (3+7+11), and Lucento (3+7), was similar. In contrast, higher disease severity was observed in plots treated with Fortix (3+11), Veltyma (3+11), and Xyway (3) plots (Fig. 1A).
- Both hybrids were found to be susceptible to tar spot disease, with P1380Q exhibiting higher disease severity compared to P1903AM. This result contrasts with the corn seed company's recommendation regarding the tar spot tolerance level of these hybrids.
  Corn yield ranged from 71.8 to 164.9 bushels/acre in New Market 1 but 152 to 230.1 bushels/acre in Blacksburg. The application of Trivapro and Adastrio (approximately 14% more than control plots) at the VT/R1 stage resulted in the highest yield followed by Fortix (approximately 7% more than control), Xyway (approximately 5% more than control plots), and the control treatment. Plots treated with Veltyma and Lucento had less yield compared to the untreated control. Interestingly, Trivapro-treated plots consistently produced higher corn yields across both 2023 and 2024, regardless of the corn hybrids planted.

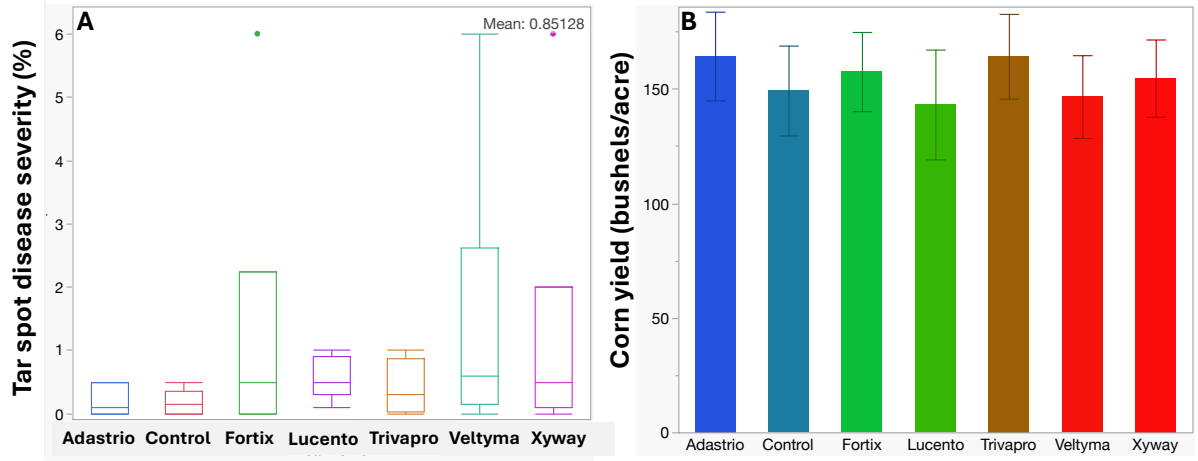

Figure 1. (A) Box plots showing tar spot disease severity (%) in plots with different chemicals compared to control plots before plant senesces. (B) Bar plots showing corn yield (bushels/acre) in plots treated with different chemicals compared to control plots.

Objective 2: We collected tar spot stromata (the fruiting body of the tar spot pathogen) from symptomatic leaves and prepared pathogen inoculum (ascospore suspension) for inoculation experiments. Corn leaves from both hybrids (**P1380Q and P1903AM™**) were wounded and treated with ascospore suspension, while leaves treated with sterile water following wounding served as controls. Treated plants were maintained in a growth chamber at 70°F and 80% relative humidity. Plants were observed daily to check for stromata development. Twenty days post-inoculation, stromata-like structures (Figure 2A; 40 x magnification) were observed on pathogen-inoculated leaves of both hybrids but not on control leaves, confirming the susceptibility of P1380Q and P1903AM to tar spot. Additionally, ascospores (Figure 2B) were identified only on pathogen-inoculated leaves under a microscope at 400 x magnification.

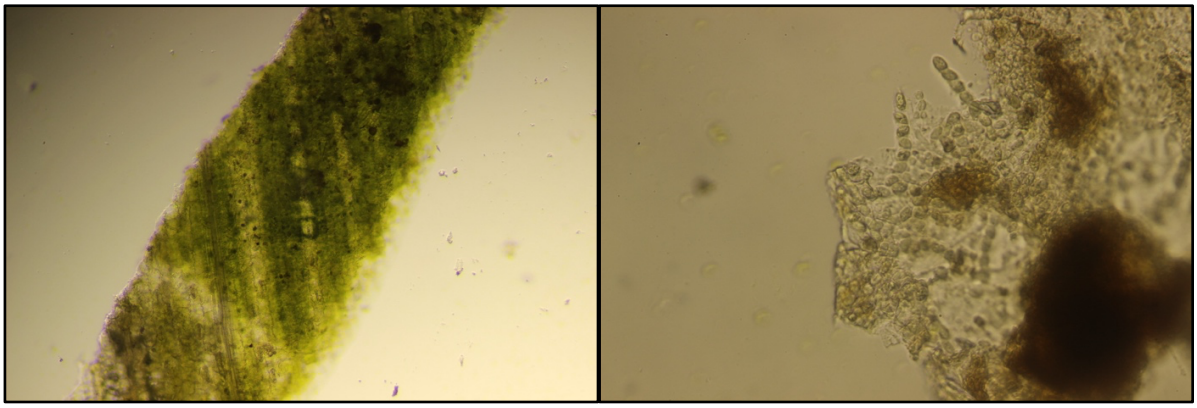

Figure 2. (A) Developed stromata (*i.e.,* black spots) were found on a leaf inoculated with tar spot pathogen and (B) ascospores of the tar spot pathogen were identified from the inoculated area of the leaves. Photos were taken 20 days after inoculation.

Objective 3:

- In our 2024 field trials, spore trap rods were collected bi-weekly from planting to harvest. In Table 2, we show that our in-house DNA sequencing and bioinformatics pipeline can effectively identify corn pathogens, including those responsible for foliar diseases and corn ear rot. Tar spot pathogen was detected in the New Market 1 location (08/15/24) but not in Blacksburg and New Market 2 locations. However, of all the corn pathogens, the gray leaf spot pathogen was found to be the most abundant on 08/15/24 in New Market 1, followed by the Curvularia leaf spot pathogen and the Anthracnose leaf blight pathogen.

Table 2. An example showing the number of unique reads mapped to corn pathogens (≥ 98% sequence identity & e-value < 1e-08) in our in-house made spore-traps.

Disease	Pathogen	Barcode 1	Barcode 2	Barcode 3
Tar spot	*Phyllachora maydis*	0	208	0
Common rust	*Puccinia sorghi*	0	248	0
Anthracnose leaf blight	*Colletotrichum graminicola*	2	3048	4
Curvularia leaf spot	*Curvularia lunata* strain W3	18	3613	30
Eyespot disease	*Aureobasidium zeae* strain KZ1	0	970	4
Gray leaf spot	*Cercospora zeae-maydis* strain LN	17	14842	15
Southern leaf blight	*Bipolaris maydis*	0	730	3
Northern corn leaf blight	*Exserohilum turcica* Et28A	0	0	0
Northern corn leaf spot	*Bipolaris zeicola* 26-R-13	18	1927	19
Southern corn rust	*Puccinia polysora*	0	0	0
Diplodia ear rot	*Stenocarpella maydis*	3	708	4
Fusarium stalk rot & ear rot	*Fusarium graminearum* PH-1	11	1526	16
Corn smut	*Mycosarcoma maydis*	0	0	0
Aspergillus ear rot	*Aspergillus flavus*	0	63	0

Barcode 1 represents spore traps collected in Blacksburg on 08/15/24.
Barcode 2 represents spore traps collected in New Market 1 08/15/24.
Barcode 3 represents spore traps collected in New Market 2 08/15/24.

- Tar spot was first observed in New Market 1 (*i.e.*, Bender1) on September 3. As an example, we plotted temperature, rainfall, and leaf wetness data recorded 14 days prior to the observation (Figure 3). While temperature trends were similar between New Market and Blacksburg, leaf wetness and rainfall varied slightly, with Blacksburg showing higher leaf wetness compared to New Market 1. Both conditions were conducive to tar spot development. However, as shown in Table 2, tar spot pathogen was not detected in Blacksburg as of 8/15/24. The absence of the

disease in Blacksburg and its presence in New Market highlights the importance of incorporating pathogen monitoring into corn foliar disease management, as fungal spores can spread via air transmission.

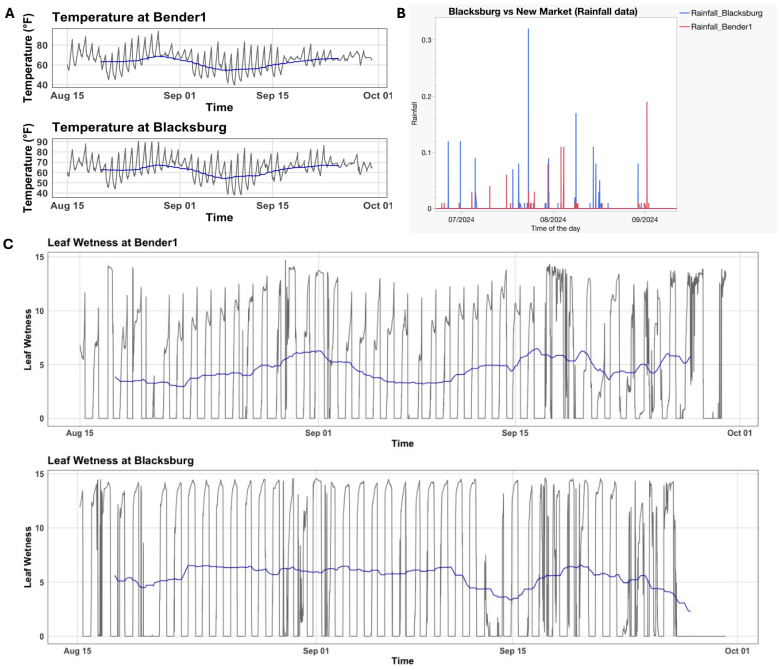

Figure 3. Comparison of (A) temperature, (B) rainfall, and (B) leaf wetness in two different locations: Blacksburg and Bender 1 (New Market).

- A correlation analysis was conducted to identify the relationship between weather variables and tar spot disease severity. Positive correlations between leaf wetness, maximum relative humidity, temperature (60°F ≤ T ≤ 73°F), leaf wetness > 7 hours, and tar spot disease severity (Figure 4);

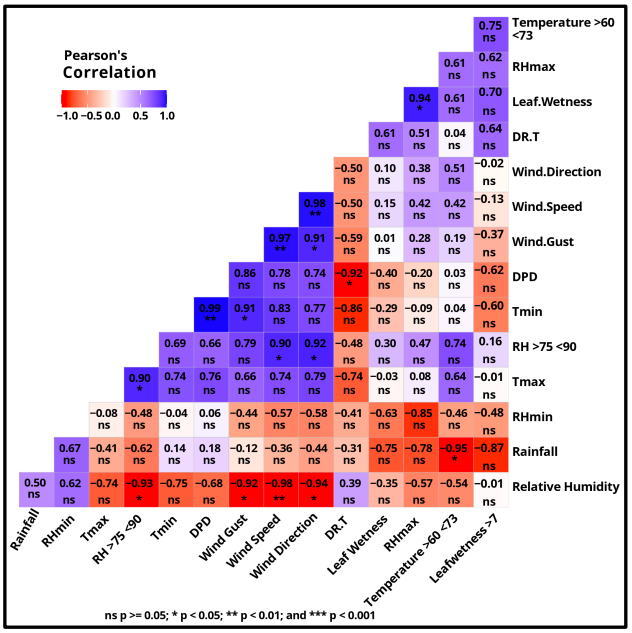

Figure 4. A heatmap showing a correlation matrix between different weather variables and tar spot disease severity.

however, these relationships were not significant. In contrast, the dew point (DPD) showed a significant negative correlation with tar spot disease severity ($P < 0.05$).

Education and Extension Activities

- Most of the project fund is used to support the Ph.D. student, Kamal Chhetri, in conducting research and extension activities outlined in the project. Kamal returned to the Southern Piedmont AREC in the 2024 Summer semester and is responsible for managing and conducting all field and lab experiments as well as data analysis. I have shared scripts of logistic regression modeling and time series analysis. The student has taken machine learning courses and developed scripts for disease forecasting. Kamal is organizing 2023-2024 data so he can develop initial predictive models. To increase the robustness of the model, we will conduct another year of field trials in the 2025 growing season.
- In 2024, we have made a point of contact with > 1K individuals (extension agents, farmers, industries, and youth) through different events to disseminate the knowledge obtained from the project, showcase in-house spore samplers and MinION sequencer, and display cellular weather station. These events include 1) The SPAREC annual field Day; 2) The annual SPAREC Family and Farm Day; 3) the School of Plant and Environmental Sciences annual symposium, and 4) the American Phytopathological Society annual meeting – Plant Health 2024.