Corn SOW for National Predictive Modeling Tool Initiative (NPMTI)

Establishing the infrastructure to develop prediction tools for diseases and mycotoxins affecting corn to better inform management decisions

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Background. Corn yield losses as a result of foliar diseases in the United States totaled more than 61 million bushels, or $2.16 billion in lost revenue, from 2014 to 2018 (Crop Protection Network Disease Loss Calculator, 2020). In addition, over $10.2 billion was lost due to grain contamination by mycotoxins (Crop Protection Network Disease Loss Calculator, 2020). However, disease-related losses vary considerably from year to year and state to state, as disease development depends on three factors: the presence and abundance of the pathogen, the susceptibility of the hybrid to that pathogen, and the environment (macro-, meso-, and micro-environment as impacted by prevailing weather conditions, agronomic practices, cropping history, canopy density, etc.) – and their interactions. Identifying risk factors (presence and abundance of inoculum, host susceptibility, favorable environmental conditions, etc.) and quantifying their associations with disease development and crop loss will enable improved management recommendations for farmers. Disease management relies on manipulating risk factors in ways that minimize infection or slow the progression of disease.

Within some cropping systems, disease-predictive models have been developed, successfully used and validated to predict the risk of disease to help guide in-season management decisions, such as application of fungicide. Currently, there are no disease risk prediction tools available for corn farmers in the U.S. preliminary models for pre-plant and in-season gray leaf spot (GLS) risk assessment were developed (Paul and Munkvold, 2004); however, these have not been implemented under current corn production systems, and additional validation across a larger production region is required. Research focusing on the development of a tar spot (TS) model (D. Smith, Personal Communication) and ear rot risk and mycotoxin models are underway (P. Paul, Personal Communication). During the 2019 and 2020 field seasons, the TS model was preliminarily programmed into a smartphone app using the Sporecaster framework. The app is under beta-testing and should be freely available for use in future seasons.

Models such as those presented above predict the likelihood of disease based largely on environmental conditions conducive for infection and disease development and assume that inoculum is omnipresent either in local infested crop residue or in the atmosphere. While such an assumption may seem reasonable based on the widespread use of conservation tillage in which previous crop residue (a source of inoculum) is left on the soil surface, corn disease risk assessment models would benefit from more direct qualifications of associations between disease severity and inoculum present and abundance throughout the growing season. Pathogen monitoring methods, including spore trapping, testing a subsample of corn residue, or sampling soil, would be invaluable for detecting early onset of plant disease (Mahlein, 2016). The National Agricultural Genotyping Center (NAGC) is able to quantify certain corn pathogens in crop residue (Pete Snyder, personal communication). In recent years, spore trapping technologies have advanced in tandem with the advances in molecular tools available for spore identification, yet their inclusion into predictive models for disease detection are vastly underutilized.

The ***rationale*** for the proposed research is that U.S. corn farmers could benefit from prediction tools for disease occurrence to improve the implementation and maximize the efficacy of management decisions and consequently reduce yield losses, ensure grain quality, and optimize profitable crop production. One shortfall of current in-season management practices such as foliar fungicides is that application may not occur at the correct time. Fungicides applied close to the onset of disease will perform at their maximum potential. Moreover, timely application of fungicides will reduce input costs, prolong fungicide efficacy by delaying resistance, and result in fewer environmental impacts of pesticides. Disease prediction tools can not only help farmers decide IF they should spray, but WHEN to spray to maximize their fungicide return on investment (ROI).

Statement of Work for 2021: Establish the infrastructure to develop prediction tools for diseases and mycotoxins affecting corn to better inform management decisions. This will be achieved through the following objectives:

Objective 1. To establish the associations among inoculum intensity, disease development, and weather in small plot trials.

Plot establishment

Experiments will be established in either (i) two no-tilled corn fields, (ii) a no-till and nearby reduced-till field, or (iii) on two separate planting dates in no-tilled corn fields in each of 9 U.S. states (IA, IN, KY, LA, MI, MO, MS, OH, WI) to investigate associations among crop residue cover (initial inoculum), airborne inoculum density, genetic resistance, and disease (gray leaf spot [GLS], northern corn leaf blight [NCLB], tar spot [TS], and Gibberella ear rot [GER]) intensity. In each experiment, half of the field will be left no-tilled while the other half will be tilled (or raked) to remove crop residue, generating high (>80% residue cover) and low (<20% residue cover) surface residue treatments (hereafter referred to as high and low residue blocks) and consequently different initial inoculum amounts. Two hybrids with different levels of resistance to the most prevalent diseases at that location (one resistant and the other susceptible) will be randomly assigned to six plots each in each residue block. Each plot will consist of eight 40 to 60-ft-long rows, spaced 30 in apart (40 in MS and LA), and planted at a recommended seeding rate. Plots in each residue block will be surrounded by a 10-ft-wide border strip (8 rows) of corn (likely the resistant hybrid) to minimize inter-plot/block interference. The layout and orientation of the residue blocks and plots within blocks will depend on field shape, size, and dimension (see

Fig. 1 for an example layout). Fields will be managed in terms of fertilizer application and weed and insect control according to standard agronomic practices for each location. Foliar fungicides will not be applied.

Sampling and data collection

Surface residue cover. Prior to planting, the amount of maize residue on the soil surface will be estimated in each residue block using a line transect method (https://www.extension.purdue.edu/extmedia/AY/AY-269-W.pdf). A 100-ft-long tape will be extended diagonally across the block and the presence/absence of maize residue will be recorded at 100 consecutive points (at 1-ft intervals) along the tape. This will be repeated in a zig-zag pattern, with at least 4 transects across each block, and the mean number of points with residue will be estimated as a measure of the percentage of the surface covered with residue.

Residue sampling. Corn residue samples, consisting of 4–6 pieces of plant material, will be collected at 20 approximately equally spaced points along the zig-zag path described above (5 samples per transect) and bulked into a single composite sample for each transect in each field. Samples will be air-dried as needed, weighed, paper-bagged, labeled, and shipped, along with the "*NAGC sample submission"* form to **National Agricultural Genotyping Center (NAGC), 1616 Albrecht Blvd N, Fargo, ND 58102**, for DNA extraction and qPCR analysis. Residue samples should be stored in a cold room until shipping to NAGC. The "*Corn RAC residue submission"* form should be filled in and filed for the research team.

Airborne spore sampling. Solar (battery)-powered Burkard High-volume Multi-Vial Cyclone Samplers will be deployed in a 20 x 20-ft clearing in the center of each residue block (labeled **B** in **Fig. 1**). Each unit will be mounted on a tripod, with the air intake approximately 3 ft above the ground. Air will be sampled at a rate of approximately 16.5 l/min, and particles will be trapped in a separate 1.5 ml vial for a 7-day period. Sample vials will be collected/replaced at weekly intervals, stored at -20C and later sent to the University of Wisconsin-Madison or Iowa State University for DNA extraction and qPCR analysis.

In addition to the Burkhard Sampler, a low-cost spore sampler (modified from Queseda et al., 2018) may be deployed in the center of each residue block. Slides attached to the spore sampler should have PCR tape attached to impact spores during the sampling period.

Airborne sampling should start as soon as possible after planting and definitely no later than V4–V5; sampling can end at R6.

DNA extraction and qPCR analysis. Protocols have been standardized by the Smith lab and can be shared with other labs. Probes developed by NAGC will be used. Serial dilutions from pure cultures, DNA extraction, and qPCR quantification will be done to construct pg DNA x spore/ml curves to enable us to quantify pathogen inoculum in terms of spore density.

Disease assessment.

(Mandatory assessments; other assessments may be done depending on the location)

Foliar disease: GLS, NCLB and TS intensity will be rated weekly or fortnightly on a minimum of 3 arbitrarily selected plants in each of the two center rows (4 and 5) of each plot. On each selected plant, the ear leaf (E), first and second leaves below the ear (E-1 and E-2), and the first, second and third leaf above the ear (E+1, E+2 and E+3) will be visually inspected from disease onset or VT (tassel) through R5 (Dent). Severity (as the

percentage of the leaf are covered with lesions of the disease in question) will be estimated directly with the aid of disease diagrams (www.cropprotectionnetwork.org). Disease incidence at each leaf position will then be calculated from severity as the number of symptomatic leaves (separate for each disease) divided by the number of plants assessed, multiplied by 100.

Ear rot: Five arbitrarily selected primary ears will be hand harvested from each plot at physiological maturity (R6), transported to the laboratory, husked, and then maintained in a greenhouse to air dry (to grain moisture of approximately 10%) until processed. Each ear will be individually rated for GER severity as percent surface area covered with characteristic symptoms of the disease (taken from Dalla Lana et al, 2020 https://doi.org/10.1094/PDIS-05-20-0952-RE).

Weather monitoring: Air temperature, relative humidity, rainfall, and surface wetness (where available) data will be collected using an available on-site weather station or a station located within a few miles of the trial location. Where possible, loggers will be programmed to collect data at 30-min intervals between planting and harvest. Remote data from IBM Weather will also be captured. Using data from two sources will ensure data quality in the early stages of this project.

Objective 2. To establish the associations among initial inoculum, disease development, and weather in commercial corn fields.

Commercial field sites. Approximately 25 commercial corn fields in each state will be identified. Each state may be stratified into 5 to 9 regions based on geography or crop productions, with 3 to 5 fields identified in each region. In each field, an arbitrarily chosen 1-acre area that does not include the headlands will be demarcated to conduct the study.

Sampling and data collection

Surface residue cover. Prior to planting, the amount of corn residue on the soil surface will be estimated in within the 1-acre using a line transect method. Percent corn residue will be recorded at 5 approximately equidistant points along each transect. At each point, a 3-foot diameter hoop will be dropped on the ground and percent corn residue within the hoop estimated visually. This will be repeated in a zig-zag pattern, with at least 4 transects across the acre (= total of 20 points). The mean percent corn residue within the demarcated 1-acre area will be calculated.

Residue sampling. Corn residue samples, consisting of 4–6 pieces of plant material, will be collected at each of the 20 approximately equally spaced points along the zig-zag path described above (5 samples per transect) and bulked into a single composite sample per field. Samples will be air-dried as needed, weighed, paper-bagged, labeled, and shipped along with a sample submission form to the PI in each respective state, who will submit the samples along with the "*NAGC sample submission"* form, to **National Agricultural Genotyping Center (NAGC), 1616 Albrecht Blvd N, Fargo, ND 58102**, for DNA extraction and qPCR analysis. Residue samples should be stored in a cold room until shipping to NAGC.

Disease assessment. GLS and NCLB intensity will be rated at approximately R5 (dent). Using the line transect method, disease will be assessed at 20 approximately equally spaced points along the zig-zag path described

above (5 sites per transect). At each site, the ear leaf (E), second leaves below the ear (E-2), and the first leaf above the ear (E+1) will be visually inspected, and severity (as the percentage of the leaf covered with lesions of the disease in question) will be estimated directly with the aid of disease diagrams. If disease is present in the upper canopy, the second and fourth leaves above the ear leaf (E+2 and E+4) also will be assessed.

Metadata. Agronomic data pertaining to each field will be collected. These data will include GPS coordinates and location (township/parish and state) of the field, cropping practices (previous crop, tillage), hybrid (maturity and resistance), fungicide product, and date of application.

Expected Outcomes. We expect to collect data that will allow us to establish the association between initial inoculum intensity (in corn residue), inoculum intensity through the growing season (spore trapping), disease development, and weather. The disease and weather data we collect will also enable us to validate existing models; for example, the Paul and Munkvold GLS model and TS model (Tarspotter v 3.0). Lastly, the data collected will resolve fundamental questions associated with the life cycle and epidemiology of the pathogens being addressed.

Pitfalls. Weather conditions may not be favorable for disease development. Since we are working in 9 states scattered across the central and eastern U.S. with varying climates and growing seasons, non-conducive weather for disease development across all states is unlikely. There are limitations associated with spore traps; for example, location of the spore traps, height above the ground, sampling rate, and efficiency (Jackson and Bayliss, 2011). Although the Burkhard spore traps outperform many other types of spore traps, optimizing their use in our research may require some adjustments to the protocol based on the data we collect.

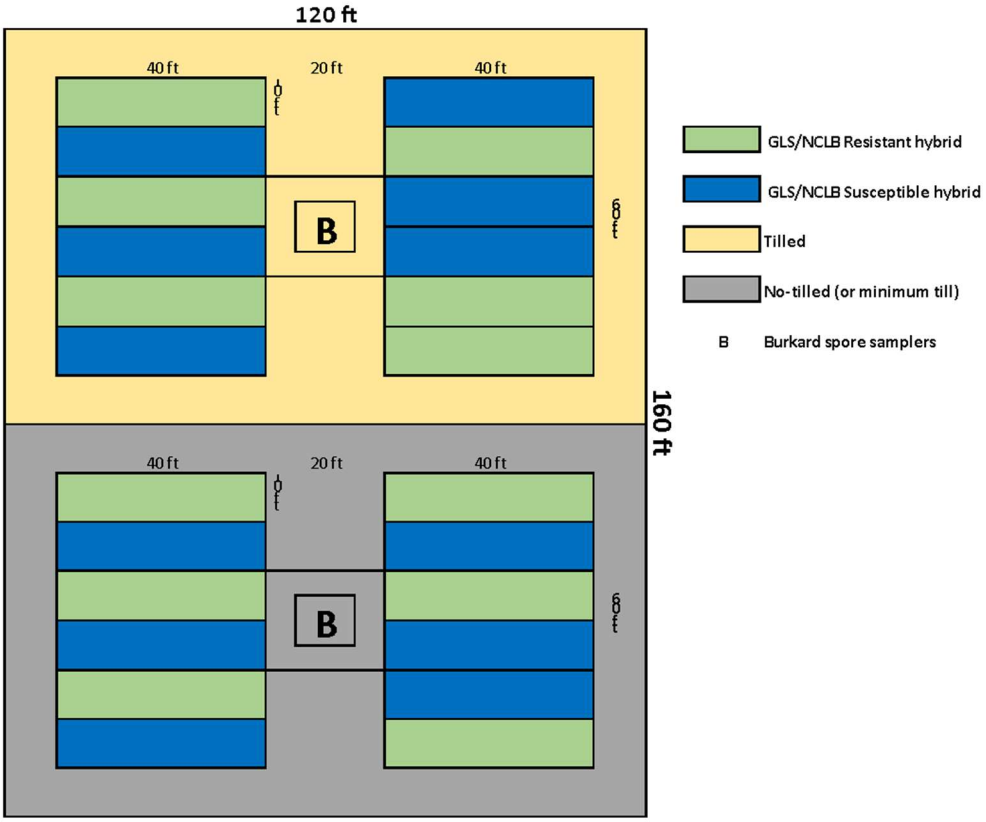

Fig. 1. Example of small plot field trial layout