Mid-year report for North Dakota Soybean Council (July 1, 2024, to November 30, 2024)

a. Research Project Title, Principal and Co-Investigators

Title: Biology and Managing Seedling Pathogens in North Dakota

Principal Investigator: Febina Mathew (North Dakota State University, Department of Plant Pathology

Co-Investigators: Joao Paulo Flores and Nitha Rafi

b. Research Overview and Objectives

Soybean (*Glycine max* L.) seedling pathogens represent a significant constraint to crop production and yield in the United States. According to the soybean disease survey conducted in 2023, Fusarium emerged as one of the most prevalent groups of pathogens infecting soybean roots during the seedling growth stage. Given the diversity of seedling pathogens affecting soybeans and the presence of favorable environmental conditions—such as wet soil and lower soil temperatures at planting—there is a pressing need for improved management strategies for farmers. A comprehensive understanding of the effectiveness of seed treatment fungicides against seedling pathogens, as well as host resistance, is imperative. Currently, there is a lack of sufficient data regarding the distribution of major seedling pathogens that infect soybeans in North Dakota. Although seed treatment fungicides are available to manage sudden death syndrome (SDS) caused by *F. virguliforme* to some extent, the impact of these products on *Pythium*, a significant seedling pathogen of soybean, remains inadequately understood. Consequently, this study aims to achieve the following objectives: (1) to investigate the distribution of microbial genera, including seedling pathogens associated with soybean roots in North Dakota; (2) to assess the impact of seed treatment on *Pythium*; and (3) to evaluate soybean accessions for resistance to *Rhizoctonia solani* and *Pythium* sp. The information derived from these studies will assist North Dakota farmers in more effectively managing seedling diseases caused by *Fusarium*, *Rhizoctonia solani*, and *Pythium* sp.

c. Completed Work: Deliverables and/or Milestones.

- *Fusarium* was identified as the most dominant genus infecting soybean roots in North Dakota, followed by *Rhizoctonia* and *Macrophomina*.
- Sudden death syndrome (SDS) was confirmed in two new ND counties, Cass and Dickey, in 2024, in addition to Richland County, where the disease was first observed in the state
- A 4% to 12% increase in the number of emerged plants was observed with the use of seed treatments against *Pythium* when compared to the non-inoculated control
- An article was published titled “Sudden Death Syndrome Identified in Soybean Fields Across Eastern North Dakota” in the North Dakota Soybean Grower Magazine for the December 2024 issue. Link - https://ndsoybean.org/wp-content/uploads/2024/11/Soybean_Grower_Magazine_Volume_13_Issue_6_-_WEB.pdf

d. Progress of Work and Results to Date

Objective 1. Characterize the species distribution of seedling pathogens associated with soybeans. (Objective in progress)

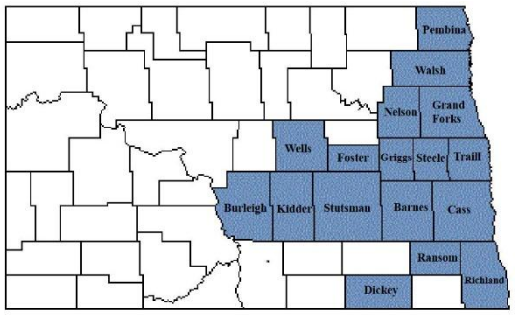

Fig. 1. North Dakota counties surveyed in 2024

Mathew's lab conducted a soybean disease survey across 17 counties in North Dakota (Fig. 1), assessing 102 fields (ranging from 2 to 13 fields per county) during the vegetative or reproductive growth stages of soybean. Soil and infected plant samples were collected from each plot to study seedling pathogens (Fig. 2) and the occurrence of sudden death syndrome (SDS). Soil samples were taken from five random locations in each field, following a 'W' pattern. These five samples were mixed homogeneously to create a single composite sample representing each field.

Fig. 2. Seedling diseases observed in a field in Wells County, ND

Diseased plants exhibiting characteristic root rot symptoms—such as brown to black root lesions, collar rot, and reddish discoloration on the roots—were collected from 80 fields to investigate various seedling pathogens (Fig. 2).

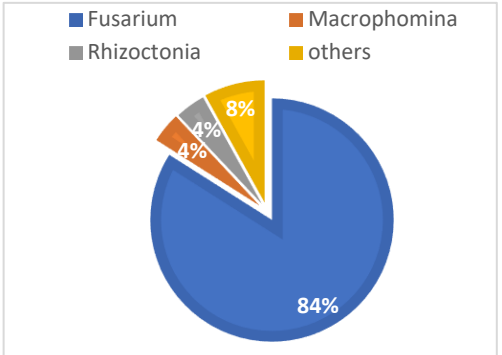

Fig. 3. Seedling pathogens associated with soybean roots from the 2024 survey (*n*= 80 fields).

For fungal isolation from the soil, roots from 7-day-old pre-germinated seedlings were placed in 20 grams of homogenized and sieved soil from each field using a rolled paper towel assay. The soybean accessions used included USDA varieties PI 561242, PI 437295, PI 468904, PI 437238, PI 243547, PI 347565B, PI 248403, PI 189866, PI 154196, PI 548504, PI 548398, PI 181536, and PI 639740. Six replications were maintained for each field, and the seedlings were incubated at 22 ± 3°C with a moisture level at 60 percent water holding capacity. After 10 days of incubation, the infected root bits were surface sterilized, blotted dry, and plated on antibiotic-amended (0.06% streptomycin sulfate) quarter-strength potato dextrose agar (PDA) media. Fungal colonies were purified using the hyphal tipping method and transferred to full-strength PDA plates for cultural and morphological characterization. The isolates were incubated at 22 ± 2°C and identified at the genus level based on morphological characteristics.

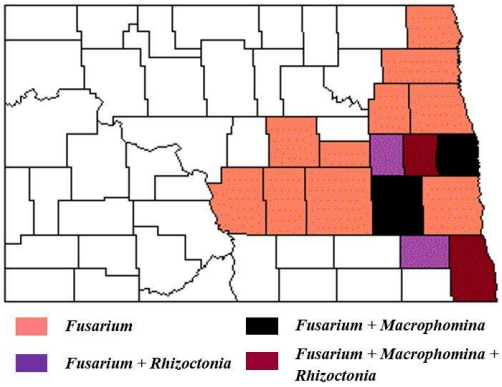

Fig. 4. Seedling pathogens from the 2024 survey by county.

A total of 458 fungal isolates were recovered from the diseased soybean roots inoculated with soil from 80 fields. Seven fungal genera were morphologically identified, including Alternaria, Cladosporium, Fusarium, Penicillium, Macrophomina, Rhizoctonia, and Rhizopus. The most predominant genus was Fusarium, which accounted for 84 percent of the total recovered isolates from the field soil (Fig. 3 and Fig. 4). Among the identified genera, Fusarium and Rhizoctonia are recognized as established seedling pathogens of soybeans.

Fig. 5. SDS foliar symptoms (leaf chlorosis and necrosis) and fungal mycelia and blue sporulation on the infected soybean roots (field in Richland County)

A total of 22 commercial fields were surveyed for the presence of sudden death syndrome (SDS) (Fig. 5) across four counties: Richland, Cass, Traill, and Dickey. Additionally, Mathew's laboratory received nine plant samples suspected of SDS (from Richland, Cass, and Dickey counties) from the Plant Diagnostic Lab (Table 1: Samples Oakes 1 to Wb) at North Dakota State University (NDSU). These plants displayed symptoms of SDS, including foliar chlorosis, necrosis, cupping/curling of leaves, severe defoliation, brown discoloration on the lower stem, and white pith with bluish spore mass on the taproot when split open, were collected from 22 fields.

We extracted DNA from the suspected plant samples (specifically roots) and conducted a quantitative polymerase chain reaction (qPCR) assay utilizing *Fusarium virguliforme*-specific primers (forward primer: 5' GTAAGTGAGATTTAGTCTAGGGTAGGTGAC 3'; reverse primer: 5' GGGACCACCTACCCTACACCTACT 3') and probe (6FAM-TTTGGTCTAGGGTAGGCCG-MGBNFQ), as described in the study by Wang et al. (2014). The positive control employed was the genomic DNA of *F. virguliforme*, while nuclease-free water served as the negative control. Each reaction comprised a total volume of 20 µl, consisting of 10 µl of TaqMan Universal Master Mix II, 0.4 µl of 10 µM forward primer, 0.4 µl of 10 µM reverse primer, 0.2 µl of 10 µM probe, 2.0 µl of DNA template, and 7.0 µl of autoclaved water. The thermal cycling conditions employed were as follows: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 1 minute. The qPCR amplifications were conducted using the QuantStudio 3 real-time qPCR machine (Applied Biosystems), and the cycle threshold (Ct) values were analyzed using QuantStudio Design and Analysis software, version 1.5.2. Among the 22 fields surveyed

for SDS, nine fields were identified as suspected based on the qPCR assay results (Table 1). A Ct value of less than 30 for a sample was considered positive for SDS, and the field was suspected of having SDS, while values exceeding 30 were considered negative for the samples and the fields. Of the nine samples received from the Plant Diagnostic Lab, six were classified as SDS suspects according to the corresponding Ct values (Table 1). We successfully isolated *Fusarium virguliforme* from the plant samples exhibiting positive Ct values and confirmed the molecular identity of a total of 28 suspected isolates by qPCR, using DNA extracted from the fungal cultures as previously described. Our study shows that SDS is expanding into new counties where it had not been previously reported. After being documented in Richland (2018) and Cavalier (2020), the disease was identified in Cass and Dickey Counties in 2024 (Figure 5). In 2024, SDS was confirmed in Richland, Cass, and Dickey Counties through the isolation of *Fusarium virguliforme* from diseased plant samples collected randomly from affected fields. The identity of the organism was confirmed using molecular assays (Wang et al. 2015).

Table 1. Details of fields surveyed in 2024 for SDS (A Ct value of less than 30 for a sample was considered positive for SDS, and the field was suspected of having SDS, while values exceeding 30 were considered negative for the samples and the fields.)

Field ID	County	Latitude	Longitude	Growth stage of soybean	Ct value (From suspected Plant DNA)	SDS Field incidence (%)	*Fusarium virguliforme* isolated and ID confirmed by qPCR?
P2	Richland	46.15245	-96.80942	R5	27.82	20	Yes
P3	Richland	46.15216	-96.79019	R5	28.30	15	Yes
P6	Richland	46.26053	-96.78165	R5	26.16	60	Yes
R7	Richland	46.262456	-96.86613	R6	26.94	5	Isolated, awaiting confirmation by qPCR
R8	Richland	46.262664	-96.86549	R6	25.70	10	Yes
R9	Richland	46.261958	-96.943551	R6	24.82	10	Yes
R10	Richland	46.262014	-97.031583	R6	34.32		No; the field was a false positive
C3	Cass	46.660001	-96.841282	R6	27.52	< 5	No
C4	Cass	46.674271	-96.841396	R6	26.12	< 5	No
C6	Cass	46.694705	-96.841066	R6	26.28	< 5	Isolated, awaiting confirmation by qPCR
TB1	Dickey				33.11		No; the field was a false positive
Oakes1	Dickey				22.87		Yes
Oakes3	Dickey				23.39		Yes
Oakes4	Dickey				24.63		Yes

Field ID	County	Latitude	Longitude	Growth stage of soybean	Ct value (From suspected Plant DNA)	SDS Field incidence (%)	*Fusarium virguliforme* isolated and ID confirmed by qPCR?
Oakes2	Dickey				Undetermined		No
Oakes5	Dickey				33.66		No; the field was a false positive
1	Richland				24.58		Yes
1	Cass				28.48		No
2	Cass				29.24		No
Wb	-				32.12		No; the field was a false positive
P1	Richland	46.153581	-96.88323	R5		0	No
P4	Richland	46.15228	-96.77705	R5		0	No
P5	Richland	46.26087	-96.81496	R5		0	No
A1	Cass	47.04373	-96.940439	R3-R4		0	No
A2	Cass	47.23882	-96.997039	R3-R4		0	No
A3	Traill	47.345589	-97.041466	R7		0	No
A4	Traill	47.381875	-97.054796	R7		0	No
C1	Cass	46.65902	-96.82561	R6		0	No
C2	Cass	46.659586	-96.825808	R6		0	No
C5	Cass	46.684257	-96.841341	R6		0	No
C7	Cass	46.712743	-96.862625	R6		0	No

Objective 2. Determine the effectiveness of fungicide seed treatments against *Pythium*. (Objective completed, the trial compromised by herbicide application)

The trial was established at North Dakota State University's Main Research Station in Fargo, ND, following complete tillage (planted on 6/10/2024). The experimental design employed was a randomized complete block design, incorporating six treatments: a non-treated control, Acceleron (comprised of Prothioconazole, penflufen, metalaxyl, and imidacloprid), Intego Suite (containing Ethaboxam, ipconazole, metalaxyl, and clothianidin), Zeltera Suite (composed of Ethaboxam, fludioxonil, metalaxyl-M, clothianidin, and Inpyrfluxam), Cruiser Maxx Vibrance (which includes Mefenoxam, thiamethoxam, fludioxonil, and sedaxane), and Cruiser Maxx APX (Vayantis) (consisting of Mefenoxam, thiamethoxam, fludioxonil, sedaxane, and picarbutrazox). Each treatment was replicated four times. Each plot measured 20 ft in length and comprised four rows with a row spacing of 15 inches. The seeding rate was set at 104,000 plants per acre. The trial was inoculated with a North Dakota isolate of *Pythium ultimum* (provided by Webster Lab) using infested wheat seeds at a rate of 120 grams per plot at the time of planting. Two weeks post-planting (06/27/2024), the trial was treated with post-emergence herbicides, specifically Liberty (glufosinate) and Select Max (clethodim). Plant emergence was assessed three weeks after planting (07/01/2024), and the number of emerged plants was compared with the non-treated control plots

using ANOVA analysis. A significant effect of treatments was not observed on the number of emerged plants ($P > 0.05$). As presented in Table 3, there were no significant differences in the number of emerged plants; however, a 4% to 12% increase in the number of emerged plants was observed with the use of seed treatments against *Pythium* when compared to the non-inoculated control.

Table 3. Effect of fungicide seed treatments against Pythium in Fargo, ND

Treatments	Plants per acre
Non-Treated	61855.2 a
Acceleron	71002.8 a
Intego Suite	69260.4 a
Zeltera Suite	66211.2 a
Cruiser Maxx APX (Vayantis)	65993.4 a
Cruiser Maxx Vibrance	64686.6 a
***P* value**	**0.571**

e. Work to be Completed.

Objective 1. Screen soybean accessions for resistance to *Rhizoctonia solani* and *Globisporangium ultimum* (formerly *Pythium ultimum*)

Mathew's lab will screen 200 accessions of soybean obtained from USDA germplasm collection for resistance to *Rhizoctonia solani* and *Globisporangium ultimum.*

f. Other relevant information: potential barriers to achieving objectives, risk mitigation strategies, or breakthroughs.

Mathew's lab was unable to complete the seed treatment trial for *Pythium* due to damage caused by the application of post-emergence herbicides (Fig. 6). As a result, the study was terminated.

Fig. 6. Pythium trial in Fargo, ND compromised by herbicide application (picture left is before the herbicides were applied)

g. Summary

A soybean disease survey conducted in the eastern region of North Dakota in 2024, encompassing 80 fields across 17 counties, revealed that the genus *Fusarium* was the predominant pathogen identified among seedling pathogens. Furthermore, sudden death syndrome (SDS) was confirmed in two new counties within North Dakota, specifically Cass and Dickey, in addition to Richland County, where the disease was first observed in the state. Several contributing factors, including increased precipitation levels in the southeastern states, minimal or no-tillage practices, cool and wet soil conditions, and the susceptibility of the host plant, may have facilitated the prevalence and spread of SDS, which was predominantly observed during the reproductive stages of the crop in the southeastern counties of North Dakota. A field trial was established to evaluate the effectiveness of various seed treatments (Acceleron, Intego Suite, Zeltera Suite, Cruiser Maxx APX [Vayantis], and Cruiser Maxx Vibrance) against *Pythium* in Fargo, North Dakota. Although no significant differences ($P > 0.05$) were observed in the number of emerged plants, a 4% to 12% increase in the number of emerged plants was noted with the application of seed treatments against *Pythium*, compared to the non-inoculated control.