2024 Pivot Bio PROVEN® 40 On Seed Viability in Varying Soil Conditions

Objective:

Develop and analyze a laboratory test to understand how varying soil conditions at planting, post planting, and up through germination can impact the viability of on-seed Proven 40 microbes.

Introduction:

Soil conditions vary across the US during planting season and can have an impact on emerging corn. Additionally, varying temperature and moisture ranges can impact synthetic nitrogen stability in the soil by initiating loss pathways for that nitrogen. Those pathways include leaching (loss through soil water); denitrification (gaseous nitrogen lost to atmosphere); and volatilization (loss to atmosphere as ammonia gas). Proven 40 On Seed (OS) contains nitrogen fixing microbes that can be applied on the seed. Once that treated seed is planted and begins germination, the Proven 40 microbes begin colonization on the radical, and continue colonization on the corn roots through plant's lifecycle. Colonized microbes then fix nitrogen at the root surface. A portion of synthetic nitrogen used in corn production can be replaced by Proven 40 microbes, which provide an environmentally stable form of nitrogen for the crop. There is a need to understand the impact varying soil conditions can have on the viability of the Proven 40 microbes during the planting through germination phase. A protocol was developed to look at six different conditions, that are commonly encountered during the spring to understand the impact on microbe viability from soil moisture and temperature variations.

Methods:

- Six environmental variations were tested in a laboratory study to simulate field conditions with varying moisture and temperatures. (Figure 1, Table 1.)
- Web Soil Survey indicates the soil used in this study is Silt Loam, further analysis indicates a pH of 6.8, with overall productive levels of fertility.
- Once seed was treated, 15 seeds per container were planted in 1-inch soil with an additional inch of soil to fill the container (12x8x7.5 cm) for the initiation of the treated evaluations. The non-treated seed was handled in the same manner as the treated seed following the same conditions and timing of the treated seed (Figure 2).
- Immediately after planting, the moisture and temperature conditions were applied to the containers.
- The "Hot &Dry" (30C) treatment was maintained via growth chamber.
- The "Warm & Moist" (21C) treatment had moisture content measured and stored under ambient conditions.
- Once applied, the treatments were kept in the targeting temperature environment, moisture was under natural evaporation based on the temperature.
- The test ran for 21 days with samples taken at day 2, 7, 14, and 21days to identify viability of the microbes on the seed.
- All enumerations were done with dilution and plating, followed by colony counting.
- Supplemental colony qPCRs were conducted for confirmation.
- No Proven 40 microbes (PV40) were detected in the non-treated check (NTC).

Figure 1. Soil moisture levels shown visibly saturated, below field capacity, and no visible moisture.

Figure 2. Seed in soil conditions, 15 seeds per treatment.

Table 1. Treatments initiated using trays containing soil set to the identified moistures and temperatures for up to 21 days.

Treatment	Soil Temp.	Soil Moisture	Soil appearance
Cold & Wet	40°F	33%	Standing water
Cold & Moist	40°F	16%	Light brown particles
Warm & Wet	70°F	33%	Standing water
Warm & Moist	70°F	16%	Light brown particles
Warm & Dry	70°F	5%	White powdery
Hot & Dry	90°F	5%	White Powdery

Results:

- Colony forming units (CFU) were present and above our minimum threshold across all environmental conditions 21 days after planting.
- The microbes replicated quickly in conditions with moisture and adequate temperatures. (Figure 3).
- The hot and dry environment depressed microbe viability, however, the counts remained above the established threshold for active nitrogen fixation. (Figure 3).
- When comparing the microbial viability across predictive nitrogen loss environments, the Proven 40 microbes remain more stable than the synthetic nitrogen. (Table 2).

Figure 3. CFU per seed over time as measured using qPCR and counting measured Day 0 to Day 21.

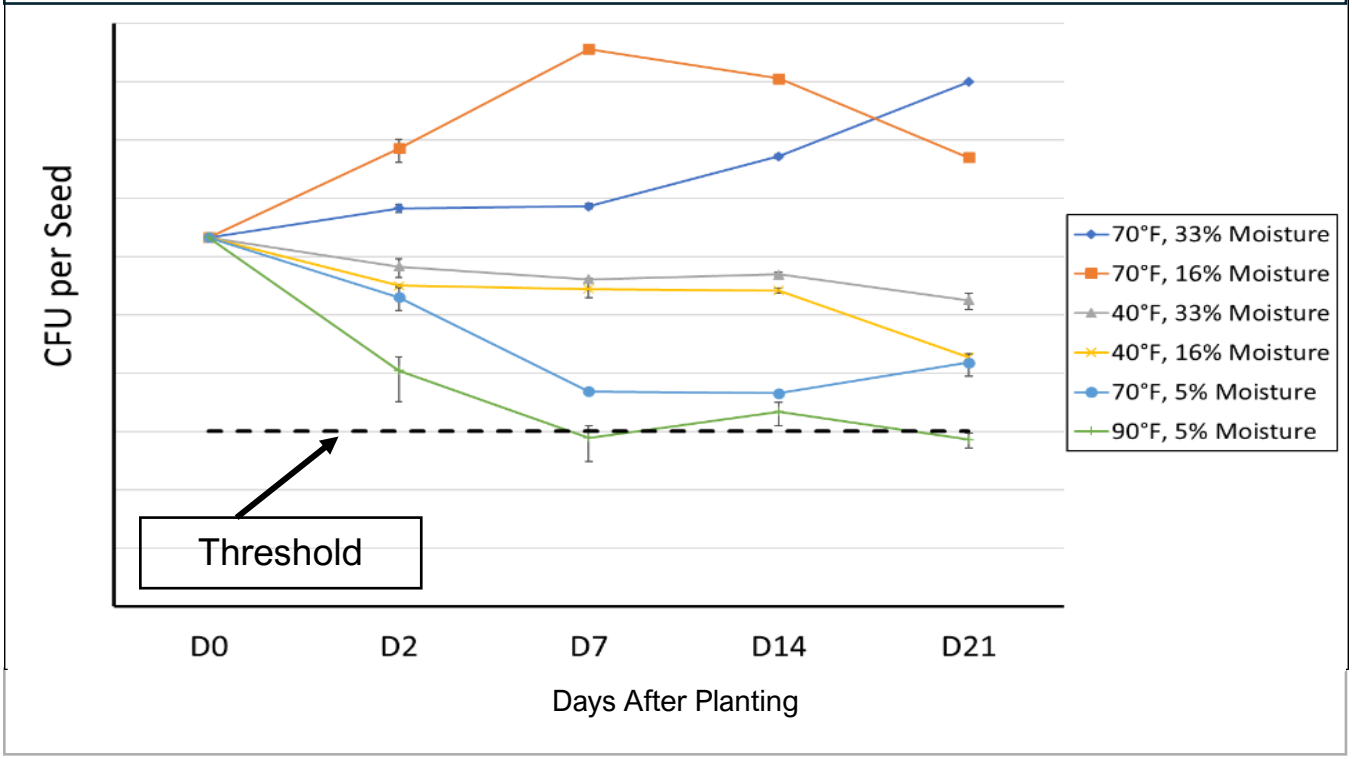

Table 2. Levels of PROVEN 40 microbes' persistence (✚) as compared to predicted levels of synthetic nitrogen loss (▬) **.**

Soil environment	PROVEN 40 OS abundancy	Synthetic Nitrogen loss caused by soil conditions		
	CFU/seed	*Denitrification*	*Leaching*	*Volatility*
Cold & Wet	✚ ✚ ✚ ✚	▬ ▬ ▬	▬ ▬ ▬	▬
Cold & Moist	✚ ✚ ✚ ✚	▬ ▬ ▬	▬ ▬ ▬	▬
Warm & Wet	✚ ✚ ✚ ✚ ✚	▬ ▬ ▬ ▬ ▬	▬ ▬ ▬ ▬ ▬	▬
Warm & Moist	✚ ✚ ✚ ✚ ✚	▬ ▬ ▬ ▬	▬ ▬ ▬ ▬	▬ ▬ ▬
Warm & Dry	✚ ✚ ✚	▬		▬ ▬ ▬ ▬ ▬
Hot & Dry	✚ ✚			▬ ▬ ▬ ▬ ▬

Summary:
The opportunity for loss of synthetic nitrogen increases when soil moisture increases and temperatures increase over 50F. Pivot Bio Proven 40 microbes fix ammoniacal nitrogen at the root surface on corn across varying soil moisture and temperature ranges. Varying moisture and soil temperature did not have a significant effect on the microbes immediately after planting and into the timing of the germination window of corn. Additionally, these microbes are active throughout the season from germination through the vegetative phase in corn and are not at risk of loss as compared to synthetic nitrogen. Therefore, the nitrogen available from the microbes is present at the actively growing root interface throughout the season, across multiple environments.