Title:
Evaluating the effect of biostimulants on the growth and yield of corn grown in normal and water-restricted conditions

Goals of the project:
The project consisted of three goals:
(1) Test the effect of 3 protein hydrolysate biostimulants on the yield and nitrogen absorption of corn subjected to different fertilization levels.
(2) Test the effect of one protein hydrolysate biostimulant on the yield of corn grown in a high tunnel and subjected to drought.
(3) Perform protein hydrolysate biostimulant on-farm tests by partnering with growers in Virginia.

Accomplishments:
The three goals of the project were met.

Goal (1)
A field test with 120 plots was conducted which enabled us to test the effect of 3 biostimulants, with two of the biostimulants at different application rates, and evaluate the yield at two fertilization levels.

Plants were sown April 21st, they reached the tasseling stage at 64 days, silking stage at 66 days, and they were harvested Sept 23rd (155 days after planting). The field was fertilized with 588 lb/A of 17-17-17 (100 lbs N/A) three days before planting, and topdressed 28 days later with either 20 or 80 lb N/A UAN.

We did not evaluate the effect of Rhodomaxx biostimulant (planned in the proposal) because we were not able to obtain the product. We obtained a protein hydrolysate from feather (abbreviated Feather Protein Hydrolysate, FPH). The protein hydrolysates tested were therefore EZGro16-0-0 (0.3 lbs N/A), Terramin (0.3 and 0.15 lbs N/A), and FPH (0.14, 0.1 and 0.07 lbs N/A). The biostimulants were applied 32 and 58 days after planting, during the vegetative stage for EZGro and Terramin, and once at day 32 for FPH. The controls were untreated plants (control). The plots were arranged according to a randomized complete block design, enabling us to have 8 replicates per treatment, increasing our statistical power.

We measured the number of leaves per plant, plant height, grain yield, SPAD index before and after the first biostimulant application, stem diameter, and the number of ears for each plot. The ear weight, length, number of rows per ear, kernels per row and kernel weight were recorded for 10 ears in each plot. In addition, we collected samples of stalks and grain of each plot for nitrogen analysis (in progress).

Goal (2)

Two parallel fields were divided into 12 plots each, for normal and drought treatment; and one biostimulant was evaluated Plants were sown April 21st, they reached the tasseling stage at 64 days, silking stage at 65 days, and were harvested September 8 (140 days after planting). The fiel d was fertilized with 588 lb/A of 17-17-17 (100 lbs N/A) three days before planting, and topdressed 28 days later with 80 lb N/A UAN.

Terramin (0.3 lbs N/A) was applied at 32 days after planting. High tunnels were moved over both fields on July 11th (81 days after planting). One field was drip irrigated every week (control), while irrigation was withdrawn for 28 days from the drought field (until August 18th). The high tunnels were removed on August 11th (112 days after planting) to allow the plants to finish their cycle. Soil moisture was recorded from July 8th to Aug 18th. We measured the number of leaves per plant, and plant height. Due to severe damage by deer, we were not able to collect data on grain yield, the number of ears per plot, or individual ear data.

Goal (3)
Co-PI Dr. Wilkinson and VCE ANR extension agents Stephen Barts (Pittsylvania County), Joanne Jones (Charlotte County), Bruce Jones (Appomattox County), and Mackenzie Gunn (Amelia County) interacted with five growers: Scott Terry and Logan Mills in Pittsylvania County, Brian Poindexter in Charlotte County, Ben Cole in Appomattox County, and Rusty Leslie in Amelia County. The growers applied Terramin at 0.3 lbs N/A on one half of the field, leaving the other half untreated. Mills, Leslie and Terry performed the trial on one field each, while Poindexter and Cole applied on two fields each. The total size of the tests ranged from 2 to 21 acres. Most of the time the biostimulant was tank-mixed with herbicides, but one producer tank-mixed with a fungicide and a foliar nutrient supplement. More details are provided in **Table 1** below.

Yields were measured as follows: (1) Leslie left 3 blocks harvested in each treated and non-treated areas (6 rows x 50 ft long), which was harvested and the yield measured by the combine from the SPAREC; (2) the yields of 1 or 2 set of 6 rows (from 160 to 1400 ft long) for Poindexter and Cole were measured using a weigh wagon; (3) the yield of Terry and Mills were measured after harvest at the weigh station.

Results:

<u>Results from Goal (1)</u>

Plot yields ranged from 11.2 to 31.2 lbs/plot (54 to 144 bu/A), with an average of 102 bu/A, much lower than the average yield of corn in the US (173 bu/A in 2023). We also did not see a large decrease in yield with decreasing fertilization: the average yields were 21.9 and 21.3 lbs/plot (105 and 100 bu/A) for the 180 and 120 N levels, respectively. The variability between plots was large, with clearly visible higher- and lower-yielding areas (**Figure 1A**). The yield data were analyzed by a mixed model in JMP 19, and the block-adjusted yield data are plotted in **Figure 1B**. No effect of any biostimulant at any rate was detected for any of the parameters we have measured so far.

A

	180 lbs N/A												120 lbs N/A											
Block identity	7	8	8	8	8	8	8	8					7	8	8	8	8	8	8	8				
	7	7	7	7	7	7	6	6	6	6	6	6	7	7	7	7	7	7	6	6	6	6	6	6
	4	4	4	4	5	5	5	5	5	5	5	6	4	4	4	4	5	5	5	5	5	5	5	6
	4	4	4	3	3	3	3	3	3	3	2	2	4	4	4	3	3	3	3	3	3	3	2	2
	1	1	1	1	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	2	2	2	2	2
Yields (lbs/plot)	25	26	26	30	31	31	29	30					30	29	21	30	29	25	26	24				
	22	20	20	22	26	27	25	25	27	27	29	29	27	25	22	22	26	23	18	24	22	21	24	25
	23	22	17	19	23	20	22	23	25	21	25	24	26	26	21	21	21	18	18	18	15	14	15	17
	20	20	16	17	19	15	15	20	22	19	21	25	23	24	22	23	21	23	21	19	21	19	17	14
	15	21	17	12	20	13	17	19	21	15	19	19	21	22	20	19	20	20	20	20	18	21	16	11

B

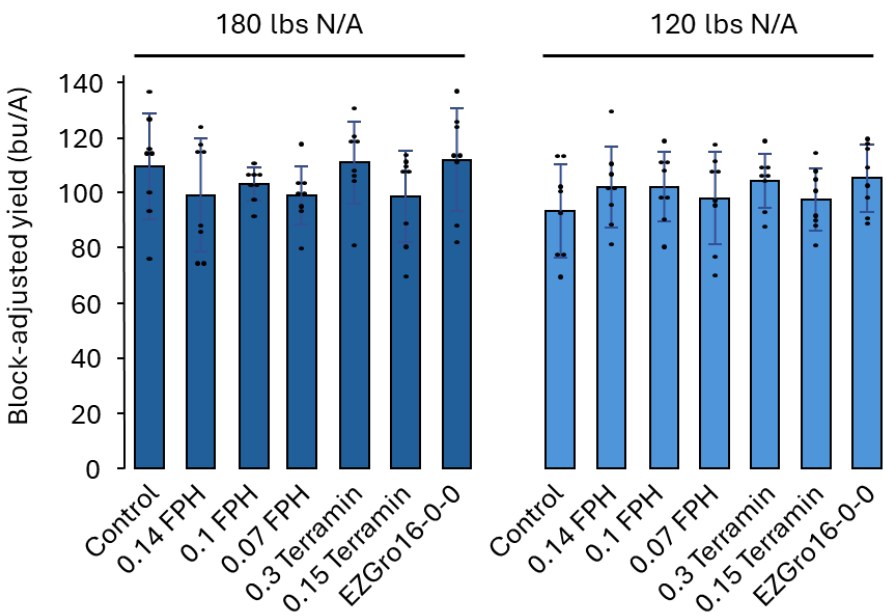

Figure 1: A. maps of the field with block location and yield per plot; each cell represents one plot. **B.** Yield results for each treatment.

<u>Results from Goal (2)</u>

Soil moisture was recorded after moving the high tunnels over the fields (**Figure 2A, 2B**). Soil moisture of all non-irrigated plots decreased steadily over the course of 28 days (**Figure 2C**), showing that drought was successfully applied. We noticed that the air inside of the high tunnels was both much warmer and much more humid than outside; condensation accumulating on the sheet of the high tunnel often dripped on the plants. This led to an unexpected phenotype, where the plants did not show typical leaf rolling, but rather purple veins (**Figure 2D**), also a symptom of drought. This translated into collapsed kernels (**Figure 2E**). We think that the high humidity and heat prevented correct kernel growth, resulting in accumulation of sugars in the leaves and stalk.

When the high tunnels were removed, the crop suffered from strong deer damage, such that we could not collect meaningful data from this trial.

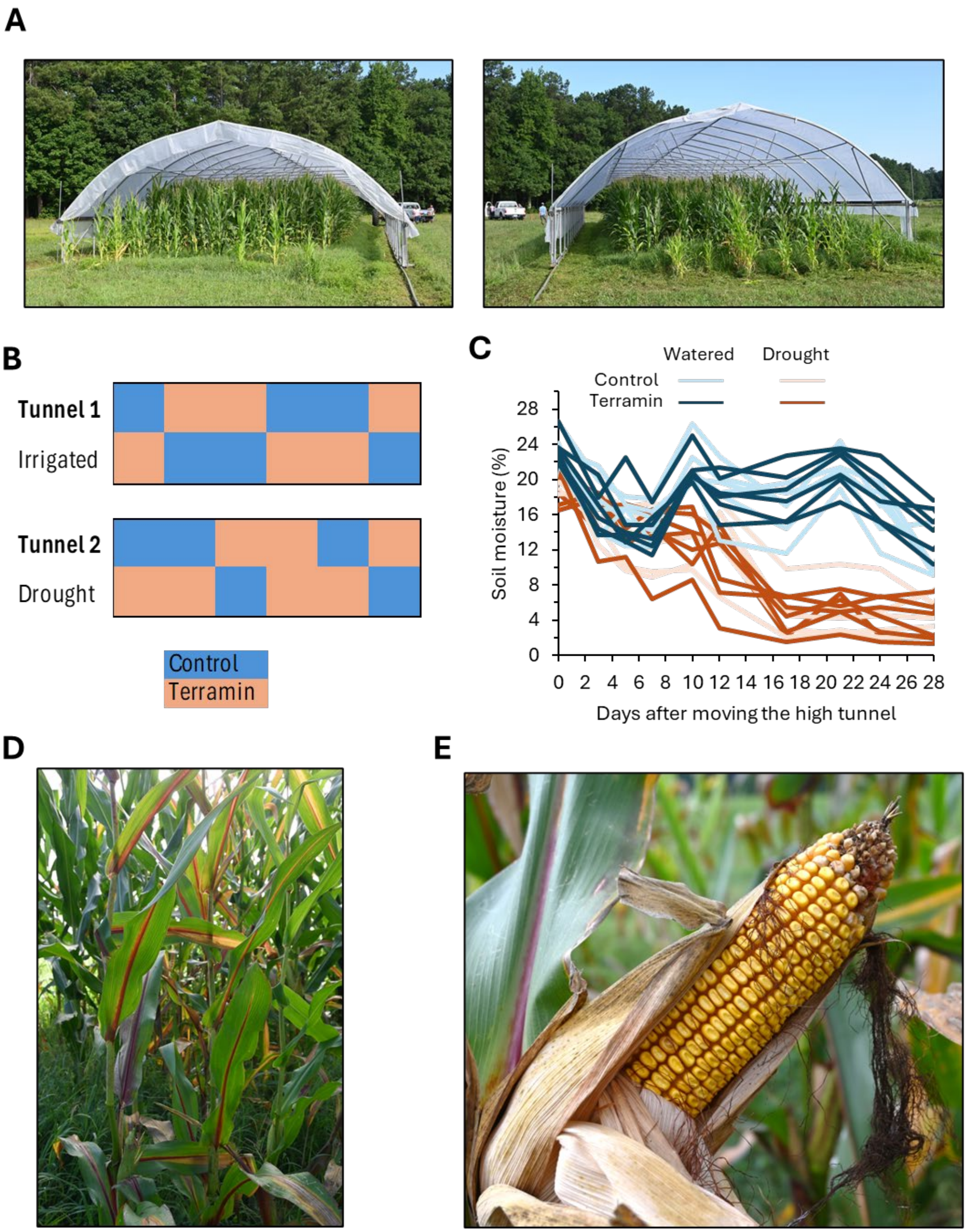

Figure 2: A. High tunnels covering each one corn field. **B:** Map of the blocks in the two fields. **C:** Evolution of the soil moisture content under the high tunnels. **D:** Heat-stressed corn plants under the high-tunnels. **E:** ear with collapsed kernels.

<u>Results from Goal (3)</u>

Out of the seven test fields, only two, from the same grower showed higher yields on the half sprayed with the biostimulant, up to 27%, and two showed lower yields (**Table 1**).

Table 1. Summary of the on-farm tests.

Grower	Yield measurement	County	Number of applications	Yield (bu/ac) Control	Terramin	% difference / control
Brian Poindexter #1	Weigh wagon	Charlotte	2	151	193.2	**27.9**
Brian Poindexter #2	Weigh wagon	Charlotte	2	169.8	178.4	**5.1**
Logan Mills	Weigh station	Pittsylvania	1	138.3	139.5	0.9
Rusty Leslie	Combine	Amelia	1	197	197	0
Ben Cole #1	Weigh wagon	Appomattox	2	216.8	216.2	-0.3
Ben Cole #2	Weigh wagon	Appomattox	2	220.9	215.3	**-2.5**
Scott Terry	Weigh station	Pittsylvania	1	151.4	148.9	**-1.7**

Discussion:

Our drought trial was partly unsuccessful because the high tunnel trapped moisture and heat, while we intended to apply only drought. It nevertheless showed that this setup can be used to apply stress on corn. The deer damage was unfortunate, preventing us from drawing any conclusions on the effect of the biostimulant on mitigating the effect of stress on the yield.

Similar to our past trials supported by the VA Corn Board, even when changing the rates and manufacturer of the protein hydrolysate biostimulants, we did not observe any effect of the products on any of the agronomical parameters we measured (Goal 1). Because there have been some reports of biostimulants being able to increase corn yield, mainly from manufacturers, we believe that this kind of product can have some benefit. We conclude that our system (RCBD with small plots, on a field that was previously fallow) does not have the power to identify any effect of the biostimulants, even when 8 replicates are employed.

Our on-farm trials were more encouraging, with two fields showing yield increases over 5%. This kind of approach has several benefits over the typical RCBD:

(1) The yield variability between plots is averaged over a large field, leading to more meaningful results.
(2) The test was conducted in genuine farm settings, where the applications, care and growth are performed by the growers.
(3) Any positive results will more likely be accepted by other growers.

It should be noted that these on-farm trials are, by conception, not replicated, and the small increase or decrease could just come from the variability between the half-fields used for the trial.

Practical Application for the growers:

Our on-farm trials showed that biostimulant application can increase yields in some cases, and some small negative impact. At a reasonable cost of 5-20$ per acre, even if they do not lead to benefit, they have the potential to increase grower's income.