TwinN trials in sugar beet, 2021 & 2022, Parma, Idaho, USA

Introduction

Trials of TwinN, a microbial biofertilizer, were performed at Parma Research and Extension Centre, University of Idaho, in 2021 and 2022 to test TwinN's capacity to produce high yields in sugar beet under reduced nitrogen fertilizer rates. The mechanisms of action of TwinN can be found at https://www.mabiotec.com/pdfs/twinn/TwinN-Mechanism-of-Action.pdf. The initial trial in 2021 produced good results and a second trial was designed and performed in 2022 to identify the optimal combination of reduced nitrogen fertilizer rate and TwinN application schedule for sugar beet production. The results are summarised here and the research reports are attached as Appendices.

2021 Trial Summary

Table 1 Yield parameters for TwinN treatments at two nitrogen rates

TRT	Notes	Conductivity mmohs	Nitrates ppm	Root Yield t/a	Sucrose %	ERS lb/a
1	Control	0.69	26.39	26.95 b	18.99	8770 b
2	Standard N – no TwinN	0.71	29.82	32.66 a	19.41 a	10,865 a
3	25% reduced N – no TwinN	0.73	27.14	34.73 a	19.18	11,387 a
4	Standard N + 1x TwinN	0.72	29.12	33.26 a	19.56	11,146 a
5	25% reduced N + 1x TwinN	0.72	25.76	33.86 a	19.34	11,214 a
6	Standard N + 2x TwinN	0.72	28.65	37.07 a	19.32	12,271 a
7	25% reduced N + 2x TwinN	0.74	26.35	37.55 a	19.12	12,264 a

Control – no fertiliser or TwinN, Standard N – 140 lb N/ac, 25% reduced N – 105 lb N/ac

Note T7 25% reduced N + 2x TwinN is MAB's recommended program

Main points

1. Root yield
    a. Standard N yield was 21.2% higher than Control
    b. Standard N + 2x TwinN yield was 13.5% higher than Standard N alone
    c. 25% reduced N + 2x TwinN yield was 14.8% higher than Standard N alone
    d. 25% reduced N + 2x TwinN yield was 8.1% higher than 25% reduced N alone

2. ERS
    a. Standard N ERS was 23.9% higher than Control
    b. Standard N + 2x TwinN ERS was 12.9% higher than Standard N alone
    c. 25% reduced N + 2x TwinN ERS was 12.9% higher than Standard N alone
    d. 25% reduced N + 2x TwinN ERS was 7.7% higher than 25% reduced N alone

Trial protocols

The trial was a randomised complete block design with six replicates

TwinN applications were at the standard rate and applied to the root zone by watering in the applied product. The first application was at 2 leaf stage and second applications in T6 and T6 were at 4-5 leaf stage. In 2022 the 3rd TwinN application was done a month after the 2nd application.

Comments

1. The highest ERSs in the trial occurred in Standard N + 2x TwinN and 25% reduced N + 2x TwinN. Although these differences were not statistically significant the ~13% yield increase was considered noteworthy.
2. The increase in ERS in T7 25% reduced N + 2x TwinN demonstrated TwinN's capacity to enable high yields at reduced N rates

2022 Trial Summary

Table 2 Yield parameters for TwinN treatments at two nitrogen rates

TRT	Notes	Conductivity mmohs	Nitrates ppm	Root Yield t/a	Sucrose %	ERS lb/a
1	Control	0.99	489	34.8	15.9	9,073
2	Standard N – no TwinN	0.96	482	33.6	15.9	8,775
3	25% reduced N – no TwinN	0.96	494	31.2	16.0	8,194
4	Standard N + 2x TwinN	0.94	503	34.6	15.9	9,097
5	25% reduced N + 2x TwinN	1.01	472	35.7	16.2	9,451
6	Standard N + 3x TwinN	1.01	517	35.0	15.6	8,925
7	25% reduced N + 3x TwinN	1.01	553	36.2	15.7	9,235

Control – no fertiliser or TwinN, Standard N – 140 lb N/ac, 25% reduced N – 105 lb N/ac

Note T5 25% reduced N + 2x TwinN is MAB's recommended program

Main points

1. Root yield
    a. Numerically all four TwinN treatments (with Standard N and 25% reduced N) produced yield increases compared to the Standard N treatment
    b. Numerically 25% reduced N alone showed a 7.1% yield decrease compared to Standard N alone
    c. MAB's recommended treatment, 25% reduced N + 2x TwinN, produced the highest yield in the trial with a 6.3% increase over the Standard N treatment
    d. 3x TwinN gave very minor yield increases over x2 TwinN at Standard N and 25% reduced N rates
2. ERS
    a. Numerically all four TwinN treatments (with Standard N and 25% reduced N) produced ERS increases compared to the Standard N treatment
    b. Numerically the 25% reduced N alone showed a 6.4% ERS decrease over the Standard N rate
    c. MAB's recommended treatment, 25% reduced N + 2x TwinN, produced the highest ERS in the trial with a 7.7% increase over the Standard N treatment
    d. The 25% reduced N rate + 2x TwinN showed a 15.3% ERS increase over the 25% reduced N rate alone
    e. 3x TwinN produced negligible or nil ERS increases over x2 TwinN treatments

Overall comments

1. 25% reduced N + 2x TwinN produced yield increases of 13% (2021) and 6.3% (2022) over the Standard N rate with reduced N costs and environmental costs
2. 25% reduced N + 2x TwinN produced ERS increases of 12.9% (2021) and 7.7% (2022) over the Standard N rate with reduced N costs and environmental costs
3. Two TwinN applications is sufficient to produce high yields at reduced N rates (compared to 3x TwinN)
4. 25% reduced N + 2x TwinN produced the highest ERS in both 2021 and 2022

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Mapleton Agri Biotec Pty Ltd

Evaluation of TwinN in Sugar Beets, 2021

Olga Walsh, PhD, Cropping Systems Agronomist

FINAL REPORT, January 28, 2022

PRELIMINARY CONCLUSIONS:

The TwinN product has shown some potential for optimizing sugar beet production. Biomass N content was maximized with one application of TwinN, with 25% reduction in N rate. While sucrose levels were comparable, double application of TwinN resulted in numerically greater root yield at both N rates, compared to plots without TwinN and plots that received one TwinN application. This suggests an opportunity to adjust N rate/TwinN combination in the future to potentially increase root yield of sugar beets. Further trials are required to confirm these initial findings. Results suggest an opportunity of improving sugar beet production with further research focused on tailoring N rates and TwinN application timing and frequency.

Table 1. Treatment structure and yield and quality, Parma sugar beet TwinN trial, 2021.

TRT	Notes	Conductivity, mmohs	Nitrates, ppm	Root Yield, t/a	Sucrose, %	ERS, lb/a
1	Control	0.69	26.39	26.95 b	18.99	8770 b
2	Prescribed N - No TwinN	0.71	29.82	32.66 a	19.41	10,865 a
3	25% less Prescribed N - No TwinN	0.73	27.14	34.73 a	19.18	11,387 a
4	Prescribed N - with 1x TwinN	0.72	29.12	33.26 a	19.56	11,146 a
5	25% less Prescribed N - with 1x TwinN	0.72	25.76	33.86 a	19.34	11,214 a
6	Prescribed N - with 2x TwinN	0.72	28.65	37.07 a	19.32	12,271 a
7	25% less Prescribed N - with 2x TwinN	0.74	26.35	37.55 a	19.12	12,264 a

The values followed by the same letters within each column indicate non-significant difference at $p < 0.05$, based on the Analysis of Variance (ANOVA) Procedure utilizing the Duncan's Multiple Range Test.

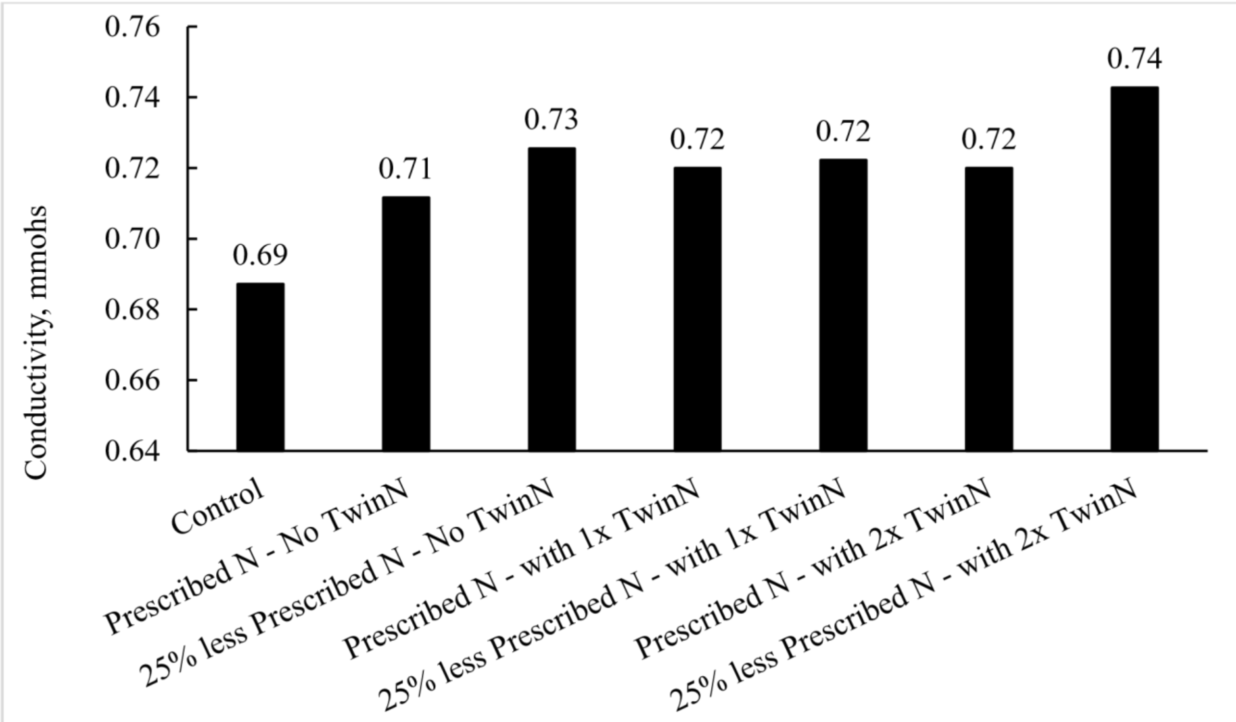

Figure 1. Conductivity as affected by nitrogen and TwinN treatments, Parma ID, 2021.

Conductivity is a measurement used to assess the quality of sugar beet roots. Typically, a high negative correlation between conductivity and fresh pulp juice purity is observed. In our study, treatments had no statistically significant effect on conductivity. All treatments had comparable conductivity values, although the unfertilized check had numerically lower conductivity (0.69), and two applications of TwinN with N rate reduced by 25% had numerically higher conductivity (0.74).

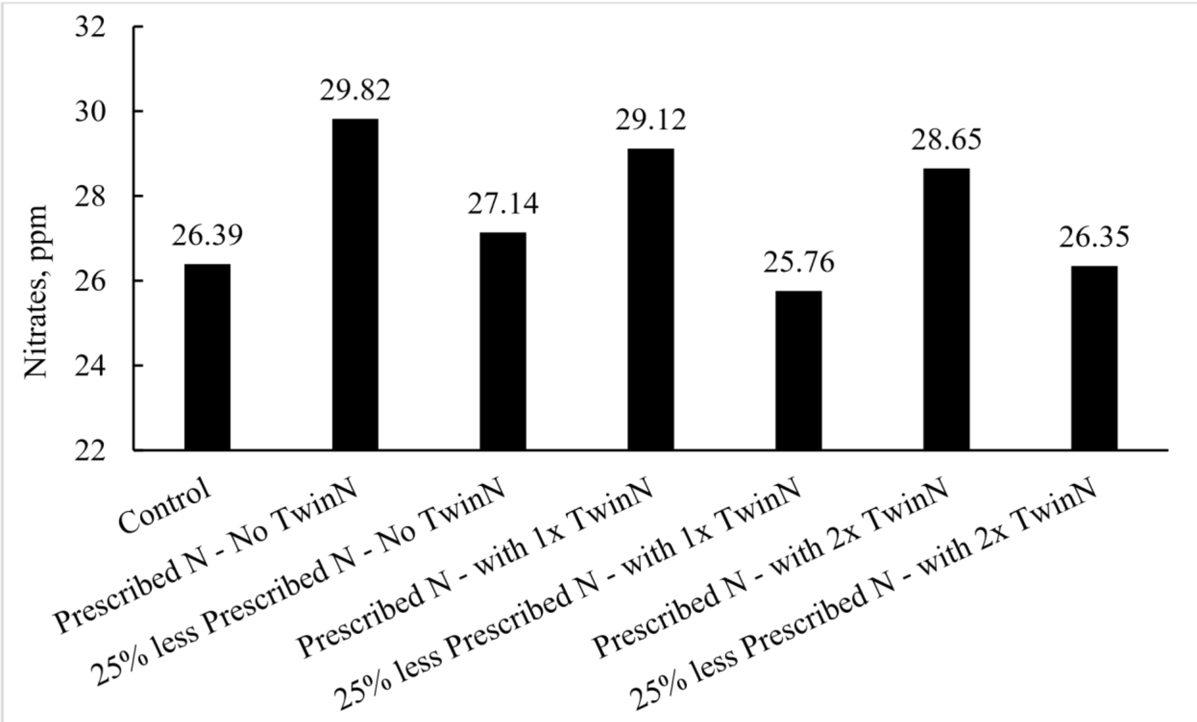

Figure 2. Nitrates as affected by nitrogen and TwinN treatments, Parma ID, 2021.

Impurities, such as nitrate N, stored in the sugar beet roots, hinder sugar extraction, which decreases the quantity of recovered sugar (ERS) from the harvested beets and increases sugar extraction costs. Concentrations of nitrate impurities in the beet roots, should not exceed 200 ppm to optimize sugar content in the beets. The sugar content tends to decrease by 0.5% for every 100 ppm of nitrates. Overall, it this trial, the nitrate levels were very low; and the differences in nitrate concentration were not statistically significant. Numerically higher nitrate levels were noted for treatments that received prescribed N rate, both with and without TwinN.

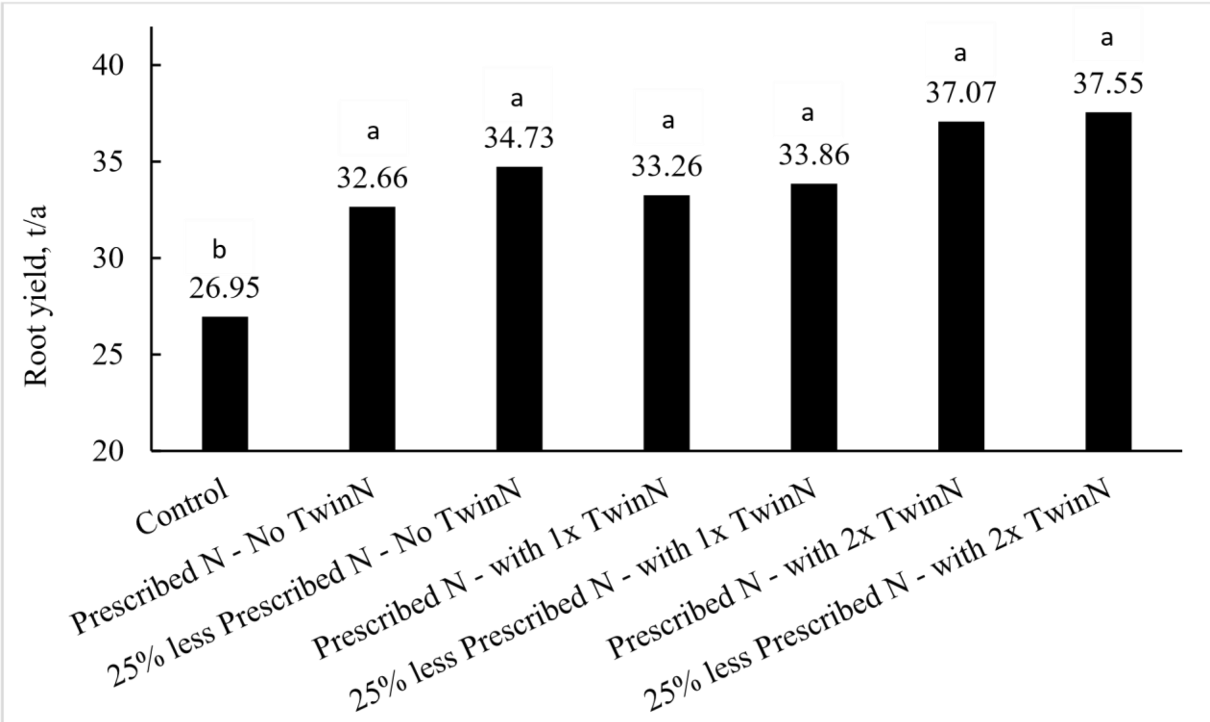

Figure 3. Sugar beet root yield as affected by nitrogen and TwinN treatments, Parma ID, 2021.

Sugar beet root yield was significantly impacted by application of N; TwinN had no statistically significant effect on root yield. The lowest root yield was noted for the unfertilized control (26.95). Interestingly, reducing N rate by 25% resulted in numerically slightly higher root yields with and without TwinN, but these differences were very minor and not statistically significant. This may be because higher N rate resulted in development of larger aboveground biomass, rather than larger beet roots, which is typical for sugar beets. Important to note, that double application of TwinN resulted in numerically greater root yield at both N rates, compared to plots without TwinN and plots that received one TwinN application. This suggests an opportunity to adjust N rate/TwinN combination in the future to potentially increase root yield of sugar beets.

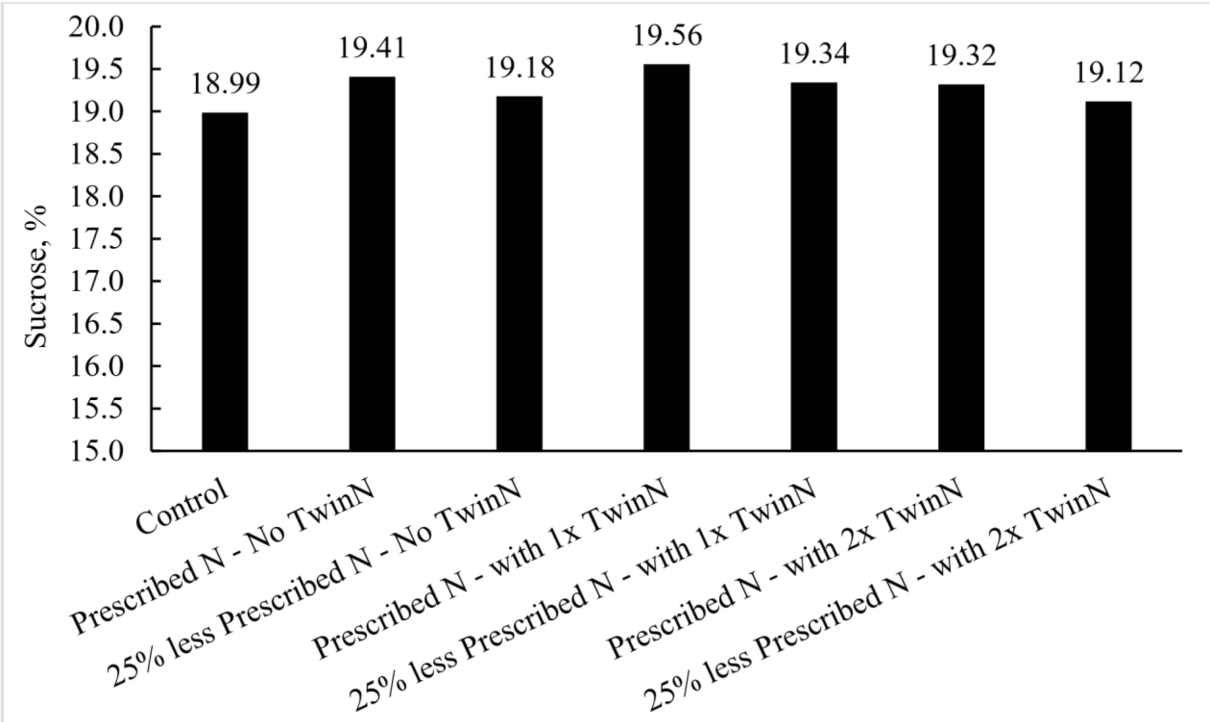

Figure 4. Sucrose concentration as affected by nitrogen and TwinN treatments, Parma ID, 2021.

Higher sucrose content in beet roots is normally associated with low-available/non excessive soil N during later growth stages. Higher N rates are typically associated with a decrease in the sucrose concentration. In this trial, there were not statistically significant differences in sucrose levels associated with N rate or TwinN application.

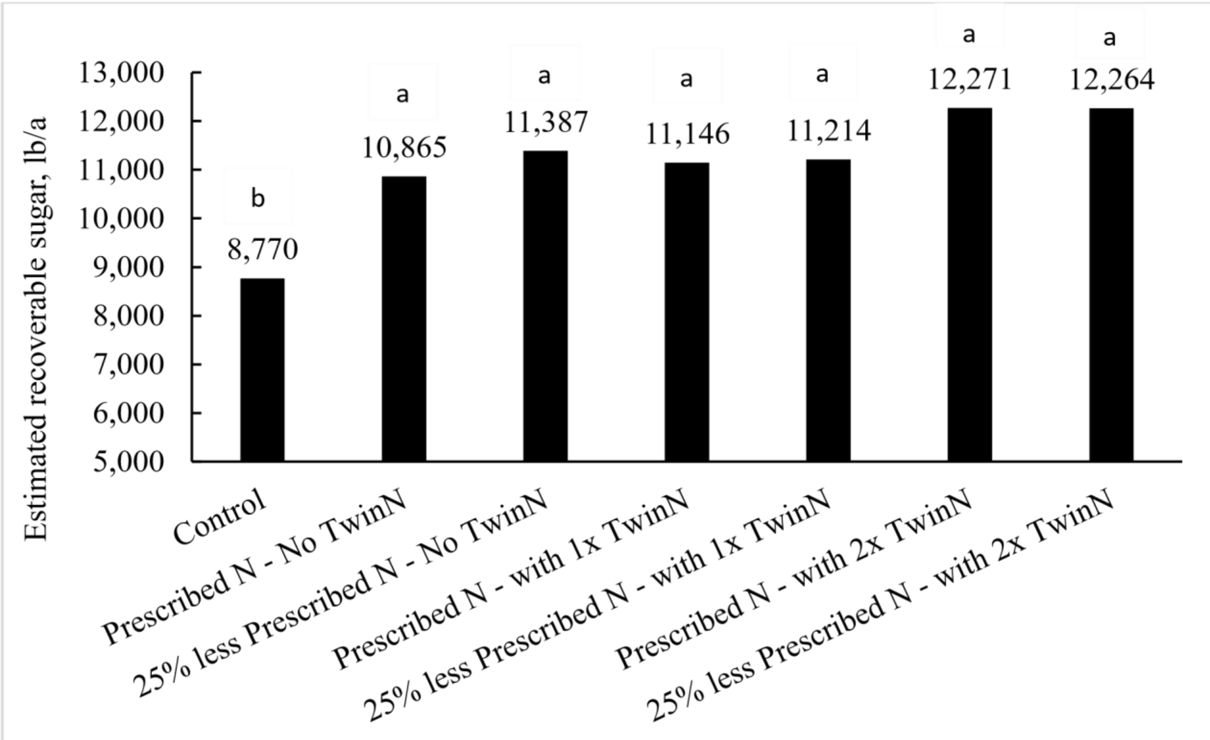

Figure 5. Estimated Recoverable Sugar as affected by nitrogen and TwinN treatments, Parma ID, 2021.

Estimated Recoverable Sugar (ERS) is a combination of root yield and sucrose content. The ERS is what ultimately matters to growers. In our trial, ERS responded to N fertilizer – the lowest ERS was obtained for the unfertilized check (8,770). However, the differences in ERS between prescribed N rate and N rate reduced by 25%, with and without TwinN were not statistically significant. This indicates that N rate could be reduced by 25% without negatively impacting ERS. Further, numerically higher ERS values were noted for double application of TwinN vs single, for both N rates (12,271 and 12,264 vs 11,146 and 11,214). This highlights an opportunity of improving sugar beet production with further research focused on tailoring N rates and TwinN application timing and frequency.

Table 2. Treatment structure and in-season crop measurements, Parma sugar beet TwinN trial, 2021.

TRT	Notes	SPAD1	NDVI1	Biomass weight1, g	Biomass N1, %	SPAD2	NDVI2	Biomass weight2, g	Biomass N2, %
1	Control	59.57 b	0.46 abc	42.94	3.97 b	51.32	0.62 b	143.05 b	2.72 c
2	Prescribed N - No TwinN	67.87 ab	0.41 c	44.89	4.73 a	52.12	0.74 a	243.55 a	3.33 ab
3	25% less Prescribed N - No TwinN	65.47 ab	0.48 ab	48.18	4.44 a	54.12	0.71 a	218.58 a	3.02 bc
4	Prescribed N - with 1x TwinN	68.32 ab	0.44 bc	42.05	4.67 a	52.35	0.72 a	245.85 a	3.40 ab
5	25% less Prescribed N - with 1x TwinN	72.58 a	0.43 bc	47.04	4.47 a	52.75	0.71 a	255.08 a	3.48 a
6	Prescribed N - with 2x TwinN	-	-	-	-	51.98	0.74 a	242.95 a	3.59 a
7	25% less Prescribed N - with 2x TwinN	-	-	-	-	54.82	0.74 a	248.08 a	3.20 ab

The values followed by the same letters within each column indicate non-significant difference at $p < 0.05$, based on the Analysis of Variance (ANOVA) Procedure utilizing the Duncan's Multiple Range Test.

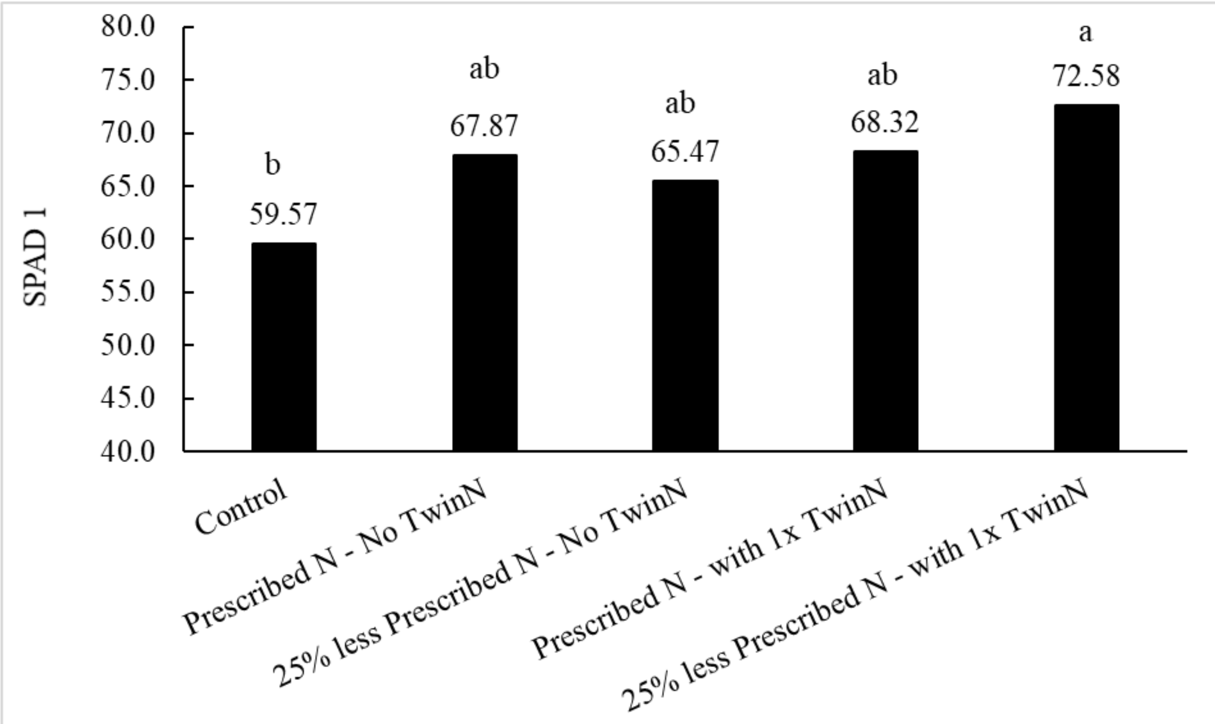

Figure 6. SPAD values 2 weeks after first TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021.

SPAD, an indirect estimate of chlorophyll content, was highest for trt 5, 25% less Prescribed N - with 1x TwinN. The lowest SPAD value was noted for the control. Other treatments were comparable in terms of SPAD values.

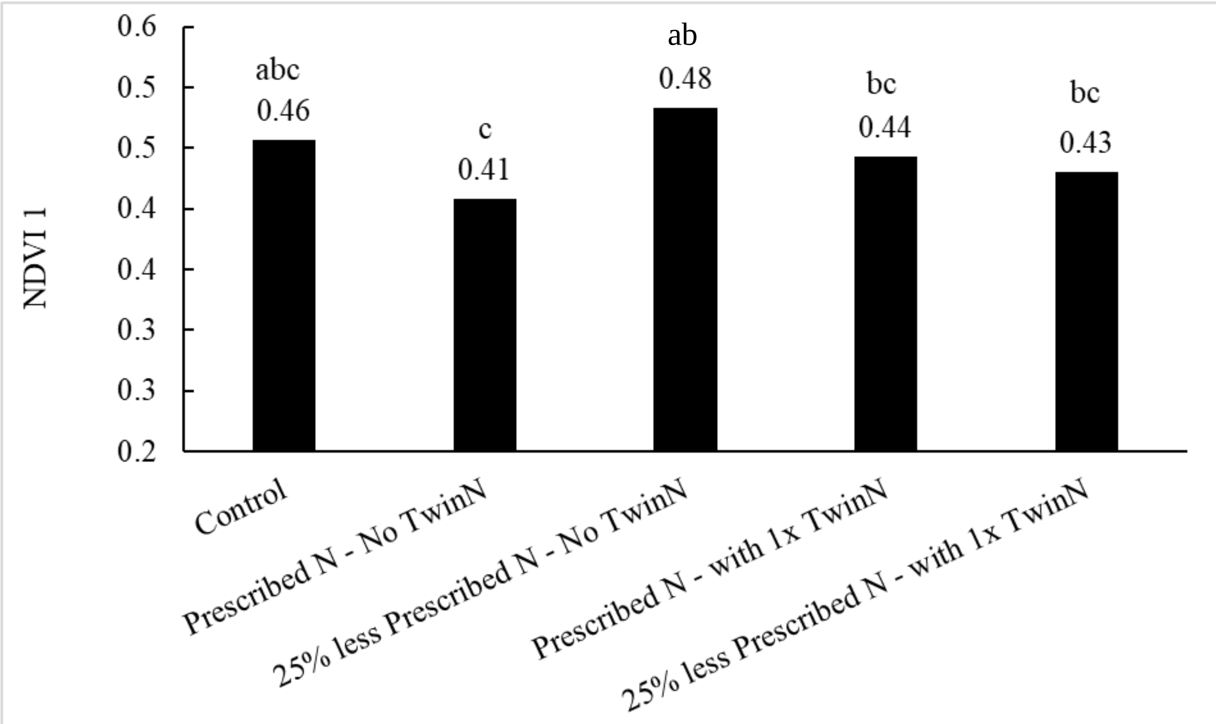

Figure 7. NDVI values 2 weeks after first TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021.

NDVI, an indirect estimate of biomass production and crop health. The NDVI values were significantly affected by treatments. The lowest NDVI was noted for trt 2, Prescribed N - No TwinN.

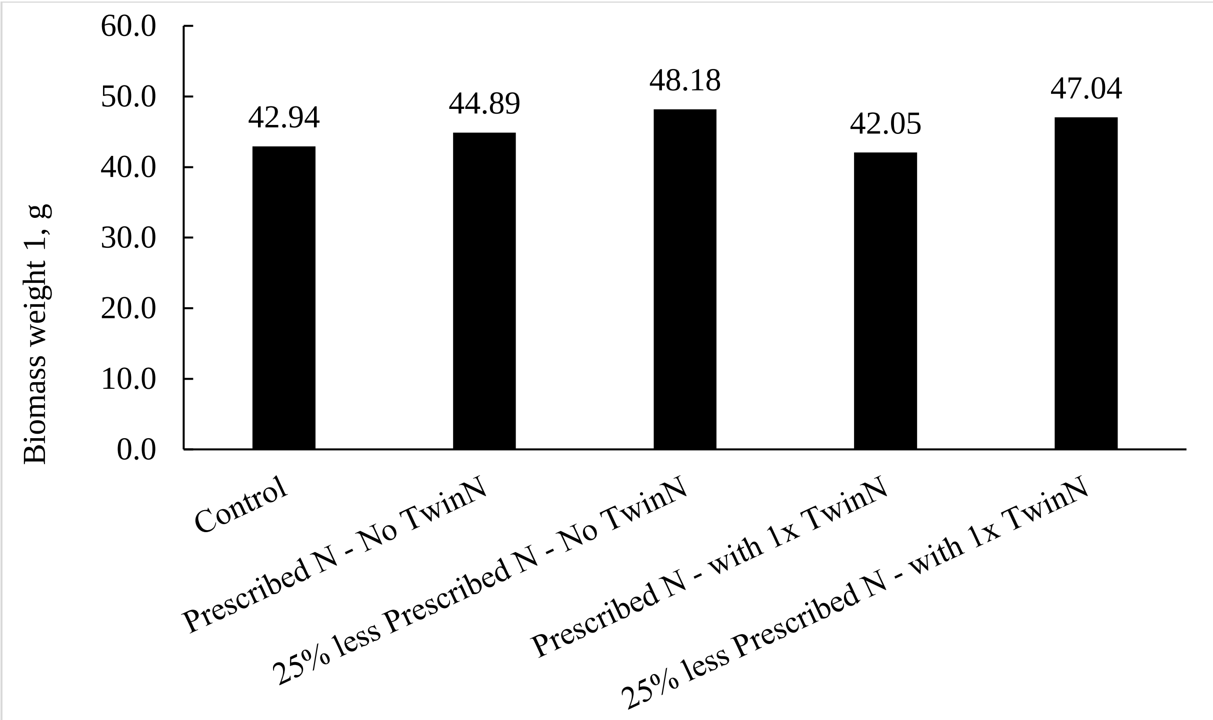

Figure 8. Biomass weight values 2 weeks after first TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021. Biomass weight was not statistically different among treatments. All treatments had comparable biomass weight early in-season.

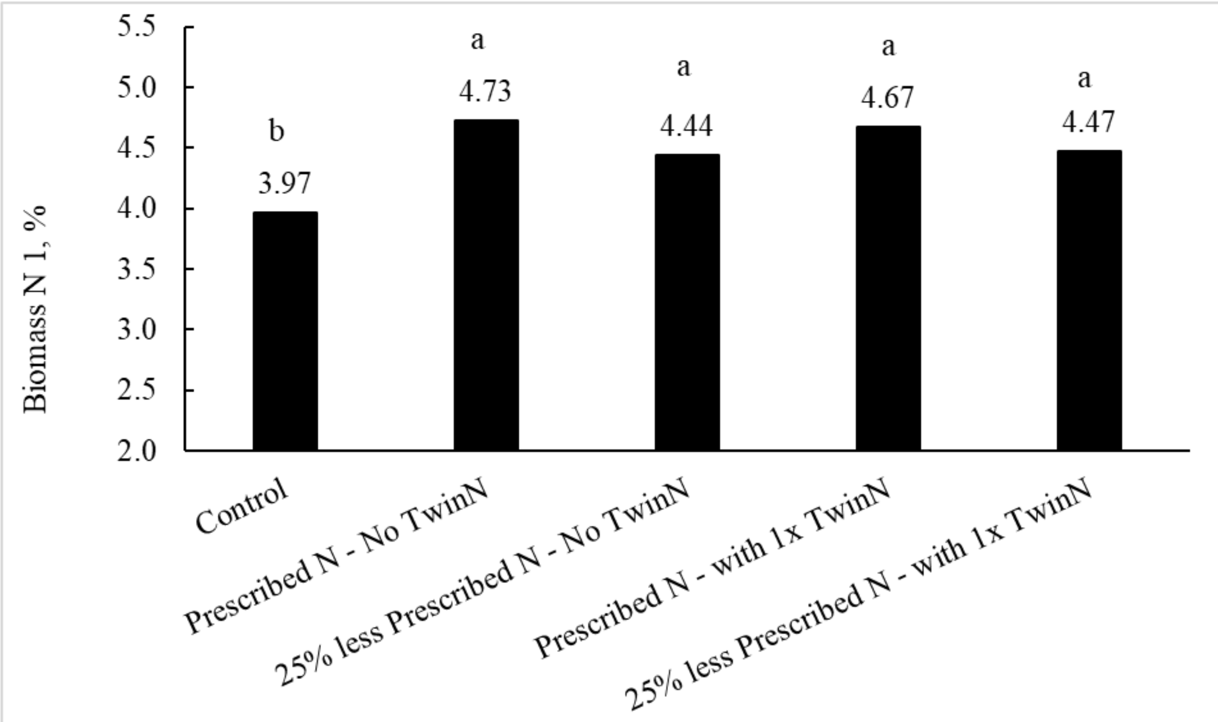

Figure 9. Biomass nitrogen content values 2 weeks after first TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021.

All treatments had significantly higher biomass nitrogen content, compared to the control. Treated plots had comparable biomass nitrogen content values.

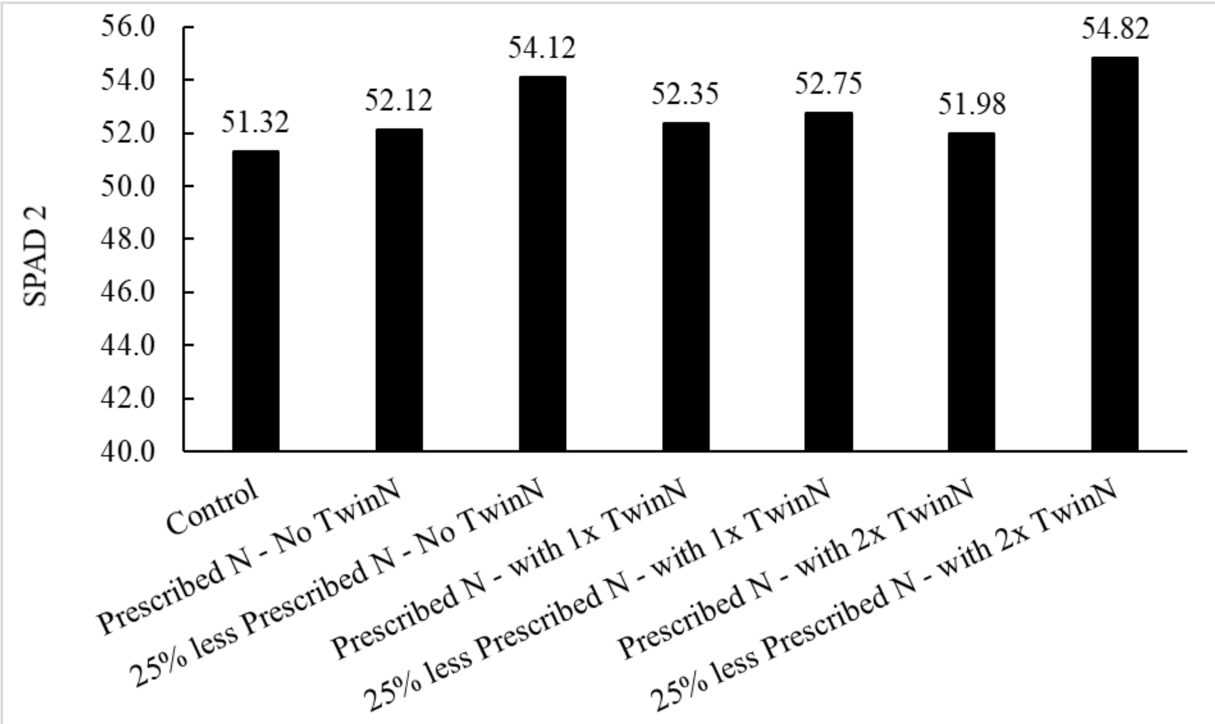

Figure 10. SPAD values 2 weeks after second TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021.

The were no statistically significant differences in SPAD values associated with applied treatments. Numerically higher SPAD value was noted for trt 7, 25% less Prescribed N - with 2x TwinN.

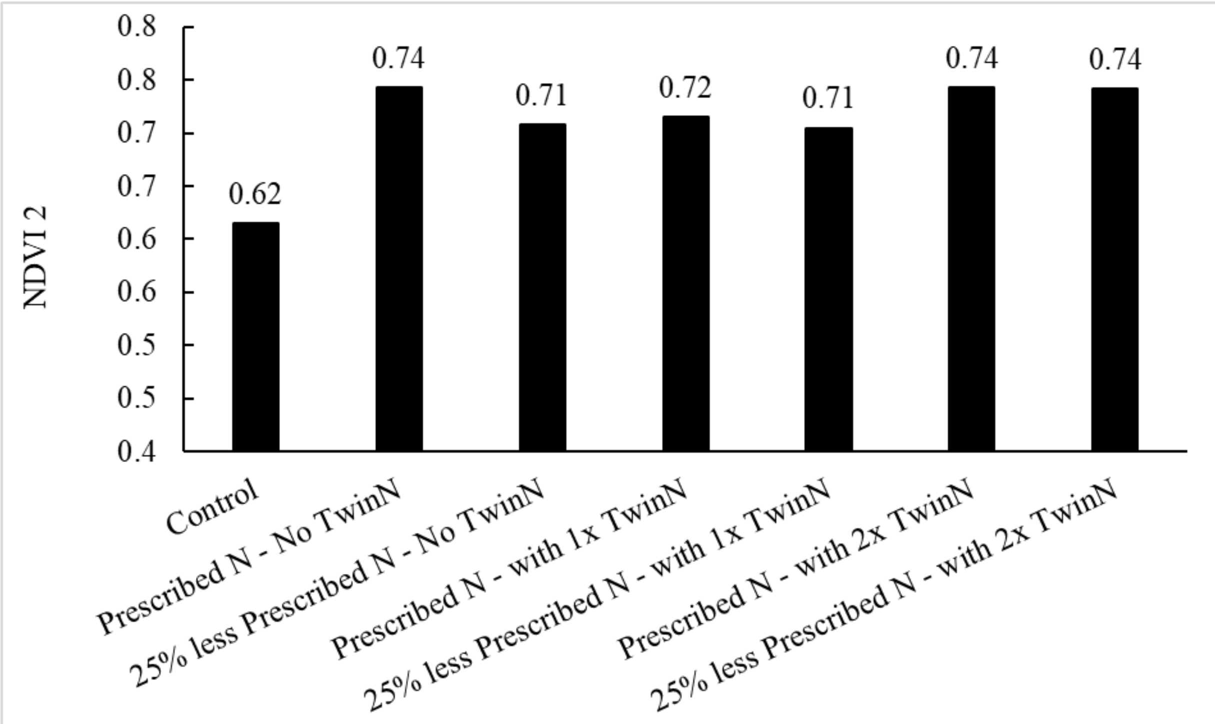

Figure 11. NDVI values 2 weeks after second TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021. All treatments had significantly higher NDVI values, compared to the control. Treated plots had comparable NDVI values.

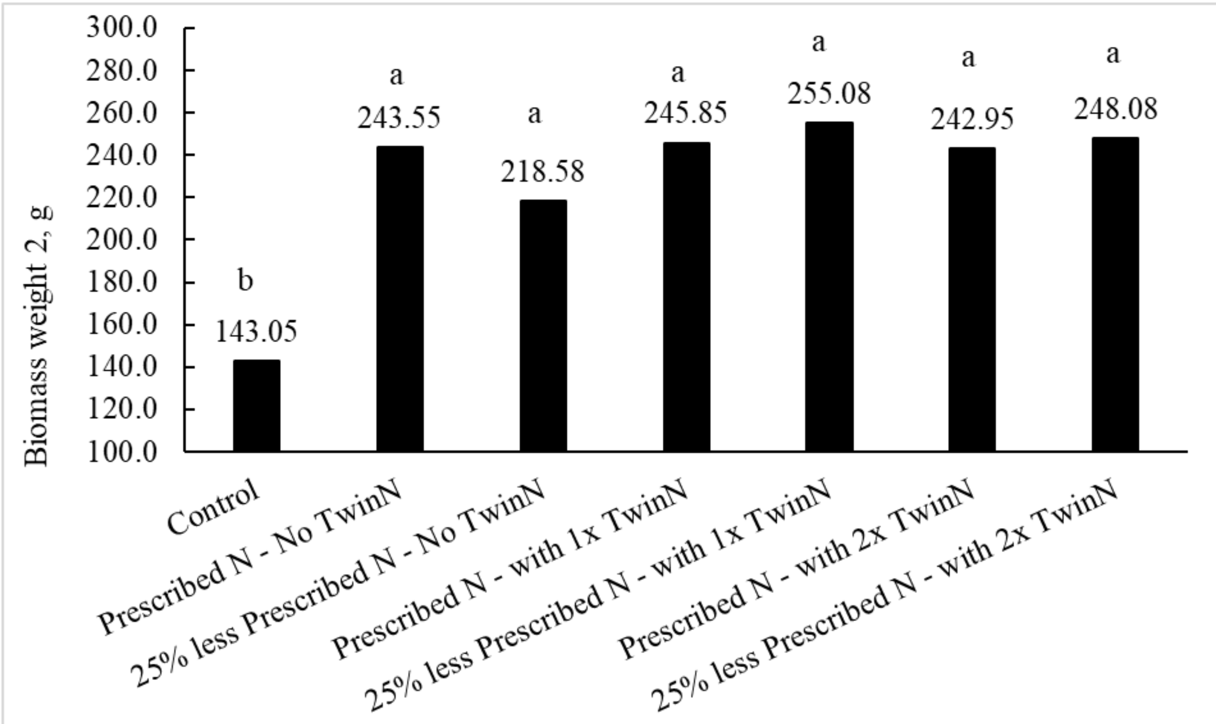

Figure 12. Biomass weight 2 weeks after second TwinN application as affected by nitrogen and TwinN treatments, Parma ID, 2021.

All treatments had significantly higher biomass weight, compared to the control. Treated plots had comparable biomass weight, although numerically higher biomass weight was observed for trt 5, 25% less Prescribed N - with 1x TwinN.

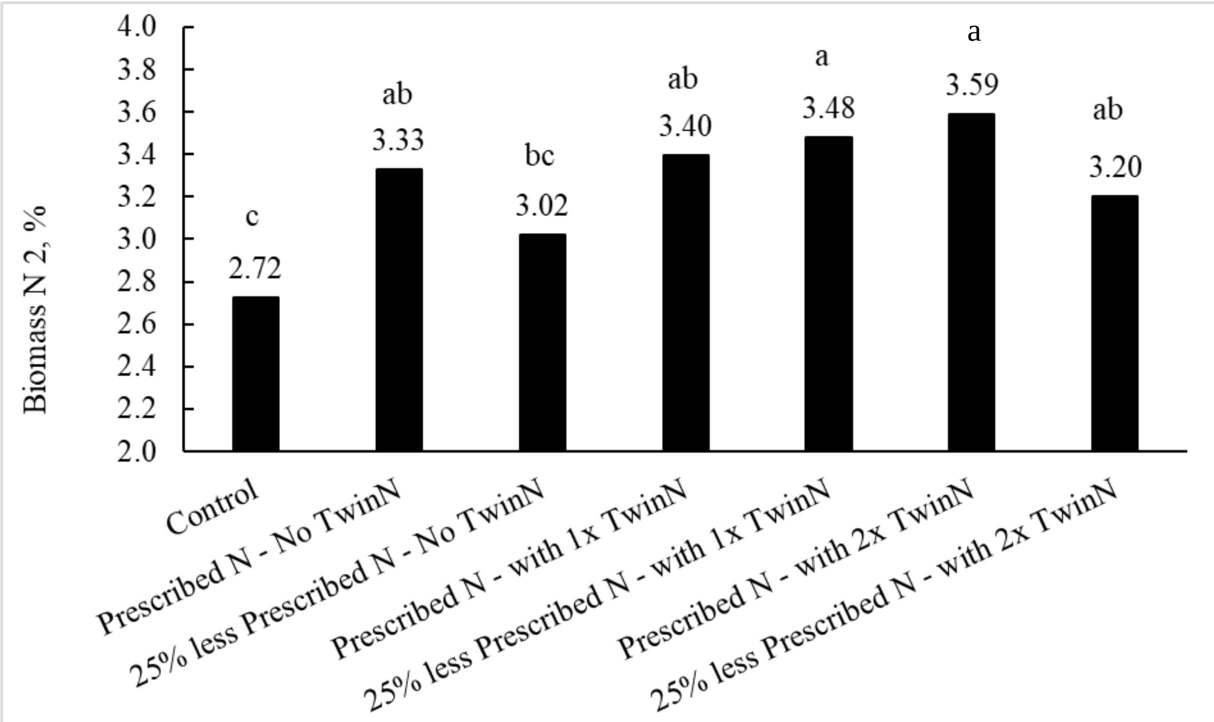

Figure 13. Biomass N 2 weeks after second TwinN application, as affected by nitrogen and TwinN treatments, Parma ID, 2021.

Biomass total N content (%) was significantly affected by both N fertilizer rate and TwinN. As expected, the unfertilized control had the lowest biomass N, compared to all other treatments (2.72). Without TwinN, when prescribed N rate was reduced by 25%, biomass N was not statistically different from that of the unfertilized control (3.02 vs 2.72). Although numerically lower biomass N was noted without TwinN, when prescribed N rate was reduced by 25%, the differences were not statistically different (3.33 vs 3.02). The highest biomass N was noted for plots that received prescribed N rate followed by two TwinN applications (3.59). This value was only statistically different from the unfertilized control and lots that received prescribed N rate reduced by 25%, without TwinN (3.59 vs 2.72 and 3.02). Overall, biomass N content was maximized with one application of TwinN, with 25% reduction in N rate. Second application of TwinN did not result in significantly higher biomass N, compared to single TwinN application (3.59 vs 3.40), whether prescribed N rate was applied or the N rate was decreased by 25% (3.20 vs 3.48).

Evaluation of TwinN in Sugarbeets, 2022

Olga Walsh, PhD, Cropping Systems Agronomist

REPORT, December 12, 2022

Summary:
The TwinN product has shown some potential for optimizing sugarbeet production. Treatment structure and sugarbeet yield and quality parameters are reported in Table 1. While sucrose levels were comparable, 2x and x3 application of TwinN resulted in numerically greater root yield at both N rates (prescribed, and 25% less), compared to plots without TwinN. Further, numerically higher root yields were achieved with trt 5 and 7, in which 25% less prescribed N was applied in combination with 2x and x3 application of TwinN. Trt 1 (control) had relatively high root yield, while trts 2 and 3 (Prescribed N - No TwinN, and 25% less Prescribed N - No TwinN, respectively) had the lowest root yields in the trial. This suggests an opportunity to 1) adjust N rate/TwinN combination in the future to potentially increase root yield and ERS of sugarbeets, while 2) simultaneously decreasing N inputs in sugarbeet fields.

Conclusion and Recommendation:
Results of the past two years suggest an opportunity for improved sugarbeet production with further research on tailoring N rates and TwinN application timing and frequency. Further field trials are required to evaluate the potential of TwinN in sugarbeets. Trials in fields with contrasting residual soil N levels are recommended.

Results:
Conductivity is a measurement used to assess the quality of sugarbeet roots. Typically, a high negative correlation between conductivity and fresh pulp juice purity is observed. In our study, treatments had no statistically significant effect on conductivity. All treatments had comparable conductivity values, although trt 4 (Prescribed N-with 2x Twin N) had numerically lower conductivity (0.94), and trts 6, 5, and 7 (Prescribed N-with 3x Twin N, and 25% less Prescribe N-with 2x and 3x Twin N, respectively) had the higher conductivity. The differences in our trial were minor.
Impurities, such as **nitrate N**, stored in the sugarbeet roots, hinder sugar extraction, which decreases the quantity of recovered sugar (ERS) from the harvested beets and increases sugar extraction costs. Concentrations of nitrate impurities in the beet roots, should not exceed 200 ppm to optimize sugar content in the beets. The sugar content tends to decrease by 0.5% for every 100 ppm of nitrates. Overall, in this trial, the nitrate levels about double the 200-ppm threshold; and the differences in nitrate concentration were not statistically significant. Numerically lowest nitrate levels were noted for trt 5 (25% less Prescribed N-with 2x Twin N.

Sugarbeet root yield was not statistically affected by the applied treatments. Numerically lower root yield was noted for trt 2 (Prescribed N - No TwinN), while the numerically higher root yields were observed for trts 5 and 7 (25% less Prescribed N-with 2x and 3x Twin N, respectively).

Higher sucrose content in beet roots is normally associated with low-available/non excessive soil N during later growth stages. Higher N rates are typically associated with a decrease in the sucrose concentration. In this trial, there were not statistically significant differences in sucrose levels associated with N rate or TwinN application.

Estimated Recoverable Sugar (ERS) is a combination of root yield and sucrose content. The same trend was observed for ERS values (Table 2). Numerically lower ERS was noted for trt 2 (Prescribed N - No TwinN), while the numerically higher ERS values were observed for trts 5 and 7 (25% less Prescribed N-with 2x and 3x Twin N, respectively). It is worth highlighting that the two higher yielding treatments with TwinN product received 25% less Prescribed N. This highlights an opportunity of improving sugarbeet production with further research focused on tailoring N rates and TwinN application timing and frequency.

Table 1. Treatment structure and yield and quality, Parma sugarbeet TwinN trial, 2022.

TRT	Notes	Conductivity, mmohs	Nitrates, ppm	Root Yield, t/a	Sucrose, %	ERS, lb/a
1	Control	0.99	489	34.8	15.9	9,073
2	Prescribed N - No TwinN	0.96	482	33.6	15.9	8,775
3	25% less Prescribed N - No TwinN	0.96	494	31.2	16.0	8,194
4	Prescribed N-with 2x Twin N	0.94	503	34.6	15.9	9,097
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6	Prescribed N-with 3x Twin N	1.01	517	35.0	15.6	8,925
7	25% less Prescribed N-with 3x Twin N	1.01	553	36.2	15.7	9,235

Based on the Analysis of Variance (ANOVA) Procedure utilizing the Duncan's Multiple Range Test.

Table 2 reports values for in-season crop characteristics (sensor measurements, and biomass values). There were <u>no</u> statistically significant differences in conductivity, nitrates, root yield, sucrose content, or the estimated recoverable sugar (ERS) at Parma in 2022. There were <u>no</u> statistically significant differences in Soil Plant Analysis Development (SPAD), Normalized Difference Vegetative Index (NDVI), biomass weight, or biomass N content at Parma in 2022 (Table 2).

Treatments were comparable in **SPAD** values. SPAD, an indirect estimate of chlorophyll content, was numerically higher for trts 2 (Prescribed N - No TwinN), and 7 (25% less Prescribed N-with 3x Twin N). The lower SPAD value was noted for trt 3 (25% less Prescribed N - No TwinN).

An indirect estimate of crop nutrient status, and biomass production is reflected in **NDVI** values. As with SPAD, numerically lower NDVI was noted for trt 3 (25% less Prescribed N - No TwinN). Numerically higher NDVI value was observed for trt 4 (Prescribed N-with 2x Twin N). Trt 1 (control) also had slightly higher NDVI, most likely due to higher weed pressure in unfertilized plots, resulting in the overall greater plant biomass volume registered by the GreenSeeker optical sensor.

Biomass weight was comparable among treatments, with numerically lower values for trts 1, 5, and 7 (control, and 25% less Prescribed N-with 2x and 3x Twin N, respectively). Trt 1, with lower sugarbeet biomass weight, while having higher NDVI supports the weed pressure suggestion above.

Biomass N content values had a negative relationship with biomass weight. Although the differences in biomass N were not significant, numerically lower biomass N was noted for trt 4 (Prescribed N-with 2x Twin N), also associated with higher NDVI, and higher biomass weight. This is expected, as higher biomass volume, reflected by high NDVI, and higher biomass volume may have resulted in dilution of N within larger leaves. There was no obvious trend on TwinN vs No TwinN treatments in biomass N content values.

Table 2. Treatment structure and in-season crop measurements, Parma sugarbeet TwinN trial, 2022.

TRT	Notes	SPAD	NDVI	Biomass weight, g	Biomass N, %
1	Control	44.0	0.76	170.4	4.9
2	Prescribed N - No TwinN	46.1	0.74	189.4	5.1
3	25% less Prescribed N - No TwinN	42.8	0.73	174.8	5.1
4	Prescribed N-with 2x Twin N	44.3	0.77	189.7	4.5
5	25% less Prescribed N-with 2x Twin N	43.3	0.75	171.5	5.0
6	Prescribed N-with 3x Twin N	43.8	0.75	173.4	5.1
7	25% less Prescribed N-with 3x Twin N	45.7	0.75	170.8	5.1

Based on the Analysis of Variance (ANOVA) Procedure utilizing the Duncan's Multiple Range Test.

Interestingly, consistent with our previous publications, **NDVI could be useful for estimation of sugarbeet yield and ERS in-season** (Figure 1).

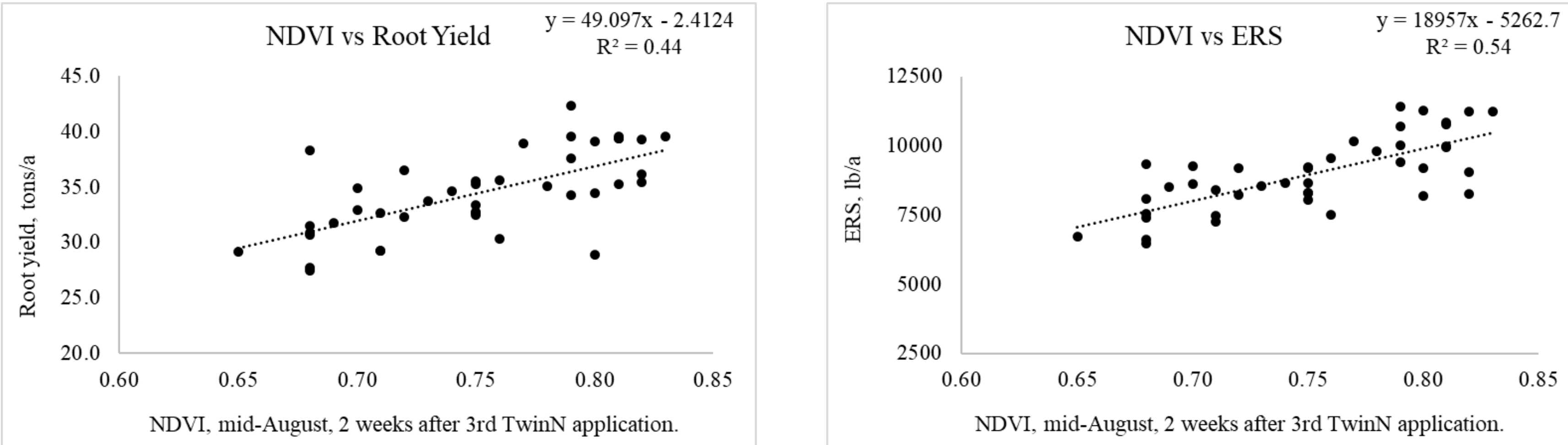

Figure 1. Relationship between Normalized Difference Vegetative Index (NDVI) measured mid-August, 2 weeks after 3rd TwinN application and sugarbeet root yield, tons/a (left), and Estimated Recoverable Sugar (ERS), lb/a, Parma ID, 2022.

Note: In 2022, the Parma farm crew has had difficulties in maintaining the weed-free growing conditions for sugarbeets in this trial. Many efforts were made to control the weeds before planting and during the growing season. Chemical sprays were done, as appropriate, by the farm crew; hand weeding was done routinely by Walsh's agronomy crew. In addition, several thousand dollars have been spent to hire field labor crews to assist hand weeding throughout the season. The higher weed pressure was observed in row 3 (of 3), which were reps 5 and 6 (of 6), thus all the plots with every treatment within those two reps were equally affected. In addition, the sprinkle irrigation was maintenance by the Parma farm crew, however several plots in the bottom right corner of the field (plots 203, 205, and 206) received relatively more water, compared to the rest of the field for about 3 weeks. We recognize that these results may/should have impacted the trial results. Unfortunately, we did not have adequate resources to precisely quantify the weed pressure or the differences in soil moisture across the field – this data may have been useful for adjusting the sugarbeet root yield values.