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Efficacy

Transit® 500

This document details the efficacy for Transit® 500, including data to support the following list of claims:

- Improves tolerance to and recovery from abiotic stress
- Increases sap Brix
- Enhances fertilizer efficiencies
- Increases nutrient uptake
- Aids the plant's ability to improve root and shoot growth and increase biomass

Transit 500 is suitable for use across all crops and geographies. The biostimulant effects of Transit 500 are highlighted by the variety of crop groupings and growing conditions shown here. Transit 500 produces a consistent biostimulant response across all crops and geographies.

A published, peer-reviewed research article (which is attached to this application, alongside its published Supplementary Information) details 21 trials supporting the abiotic stress mitigation claim. This paper highlights that Transit 500 improves the tolerance to and recovery from abiotic stress, including heat, drought, cold/frost, excess moisture, and salinity. The trials, where stress conditions were either simulated or experienced under field conditions, were conducted on corn, wheat, soybean, sugarcane, tomatoes, peppers, strawberries, avocados, and lentils. During the statistical analysis of the data obtained in these studies, a p value of 0.10 was utilized for all trials. Under the guidance of ISO/TC 134 (Fertilizers, soil conditions, and beneficial substances), a p value of 0.10 can be used under controlled conditions and a p value of 0.15 can be used under field conditions. This manuscript describes trials under both controlled greenhouse conditions and field conditions, so a p value of 0.10 was used for all, for consistency. Most of the trial data is included in the manuscript or supplementary information, but any additional data can be provided upon request. The citation for this publication is:

Sleighter, R.L.; Hanson, T.; Holden, D.; Richards, K.M. Abiotic stress mitigation: A case study from 21 trials using a natural organic matter based biostimulant across multiple geographies. *Agronomy*, 13, 728, 2023. DOI: 10.3390/agronomy13030728

This manuscript focusing on abiotic stress mitigation also provides data to corroborate the claims of increasing sap Brix and enhancing fertilizer efficiencies, which aid the plant's ability to improve root and shoot growth and increase biomass. A summary of the trials within the publication, specifically relating trials to claims with noted statistically significant differences between treatment groups, is as follows:

- Tomato plants grown in a nursery in sandy-loam soil under drought stress had statistically significant increases in plant and shoot weights during stress and statistically significant increases in plant, root, and shoot weights after drought stress when Transit was present, in comparison to stressed untreated controls without Transit. This randomized complete block design (RCBD) trial had 8 replicates for the 3 treatments (unstressed untreated control, stressed untreated control, and stressed treated with Transit), and data was evaluated using one-way analysis of variance (ANOVA) to determine if measurements were statistically different ($p=0.10$). When $p<0.10$ from ANOVA, a post hoc multiple comparison test was performed using least significant difference (LSD) to ascertain which averages were different. See Figure 1 in Sleighter et al., 2023, where error bars represent one standard deviation (and Table S1 in its Supplementary Information).

- Tomato plants grown in a nursery in sandy-loam soil under salt stress had statistically significant increases in plant, root, and shoot weights when Transit was present (for both soil and foliar applications), in comparison to stressed untreated controls without Transit. These 2 RCBD trials each had 8 replicates for the 4 treatments (unstressed untreated control, stressed untreated control, unstressed treated with Transit, and stressed treated with Transit), and data was evaluated using one-way ANOVA to determine if measurements were statistically different ($p=0.10$), and when they were, a post hoc multiple comparison test using LSD was performed. See Figures 2-3 in Sleighter et al., 2023, where error bars represent one standard deviation (and Tables S2-S3 in its Supplementary Information).
- Cumulative yields from 17 pick days during the season showed that yields from strawberry plants, grown in 2 L containers in a commercial soilless media, that were treated with Transit plus the grower standard (GS) fertilizer program and under salt stress matched the yields from unstressed GS controls (without Transit), which were much higher than (approximately double) the yields from stressed GS control plants that did not receive Transit. This RCBD trial had 6 replicates for the 3 treatments (unstressed GS control, stressed GS control, and stressed treated with Transit + GS). See Figure 4 in Sleighter et al., 2023.
- Pepper plants under drought stress (drought induced either pre-bloom or at bloom) had statistically significant increases in yield when Transit was present alongside the grower standard (GS) fertilizer program, in comparison to stressed GS controls without Transit. Also, pepper plants without stress had statistically significant increases in yield when Transit was present alongside the GS fertilizer program, in comparison to unstressed GS controls without Transit. This RCBD trial had 6 replicates for the 6 treatments (unstressed GS control, unstressed GS + Transit treatment, stressed GS controls during drought at 2 different times, and stressed GS + Transit treatments during drought at 2 different times), and data was evaluated using one-way ANOVA to determine if measurements were statistically different ($p=0.10$), and when they were, a post hoc multiple comparison test using LSD was performed. See Figure 5 in Sleighter et al., 2023, where error bars represent one standard deviation (and Table S4 in its Supplementary Information).
- Yields from avocado trees under combined drought and salt stress that were treated with Transit alongside the grower standard (GS) fertilizer program were 45% higher than stressed trees only receiving the GS (without Transit). Absent the stress, the trees that were treated with Transit alongside the GS fertilizer program were 24% higher than trees only receiving the

GS (without Transit). This RCBD trial had 6 replicates for the 4 treatments (unstressed GS control, stressed GS control, unstressed GS + Transit treatment, and stressed GS + Transit treatment), and data was evaluated using one-way ANOVA to determine if measurements were statistically different (p=0.10), and when they were, a post hoc multiple comparison test using LSD was performed. See Figure 6 in Sleighter et al., 2023, where error bars represent one standard deviation (and Table S5 in its Supplementary Information).

- From 11 in-field side-by-side corn trials conducted over the course of 3 years, yields from corn under either cold and wet stress conditions or hot and dry stress conditions (depending on the year) that were treated with Transit alongside the grower standard (GS) fertilizer program were 5-21% higher than stressed corn only receiving the GS (without Transit). The yield increases were highest in the fields experiencing more stress. In addition, larger and deeper roots were observed for the corn receiving Transit alongside the GS fertilizer program, where there was a 7-88% increase in root profile width, with the largest increases occurring at the deeper parts of the soil profile. Only 1 of the 11 trials showed a yield decrease when the biostimulant was present. See Table 1 and Figure 7 in Sleighter et al., 2023.
- Yields from soybean that was grown in a medium texture sandy clay soil under heat and drought stress that were treated with Transit alongside the grower standard (GS) fertilizer program were 18% higher than stressed soybeans only receiving the GS (without Transit), which was a statistically significant difference. In addition, statistically significant increases in stomatal conductance and antioxidative enzyme activity were observed for the soybeans receiving the Transit alongside the GS fertilizer program. This RCBD trial had 4 replicates for the 2 treatments (GS control and GS + Transit treatment), and data was evaluated using one-way ANOVA to determine if measurements were statistically different (p=0.10), and when they were, a post hoc multiple comparison test using LSD was performed. See Table 3 in Sleighter et al., 2023.
- Yields from sugarcane that was grown in a medium texture sandy clay soil under heat and drought stress that were treated with Transit alongside the grower standard (GS) fertilizer program were 30% higher than stressed sugarcane only receiving the GS (without Transit), which was a statistically significant difference. In addition, increases in stomatal conductance and antioxidative enzyme activity (some to a statistically significant degree) were observed for the sugarcane receiving the Transit alongside the GS fertilizer program. This RCBD trial had 4 replicates for the 2 treatments (GS control and GS + Transit treatment), and data was evaluated using one-way ANOVA to determine if measurements were statistically different (p=0.10), and when they were, a post hoc multiple comparison test using LSD was performed. See Table 3 in Sleighter et al., 2023.
- Wheat was grown in Vectis (Victoria, Australia) in a grey clay soil type, where the previous crop was canola, and the wheat was sown into mulched canola stubble. Rainfall during the season was well below average, and significant frost events occurred in early-mid August (see Table 1 below). Yields from wheat under combined drought and cold stress that were treated with Transit alongside the grower standard (GS) fertilizer program were 34% higher than

stressed wheat only receiving the GS (without Transit), which was a statistically significant difference (Table 2 below). Considerable frost events occurred about 1 week prior to treatment and 1-3 days after treatment, which was also when rainfall began to decrease, significantly affecting crop yields in the region. Statistically significant increases (18-53%) in sap Brix were observed for the wheat receiving the Transit alongside the GS fertilizer program, which was sustained from 1-52 days after treatment. These measurements were taken during the highest stress points of the season, when conditions were cold and there was little rainfall, as well as when temperatures were beginning to rise during the Australian spring. This RCBD trial had 3 replicates for the 2 treatments (GS control and GS + Transit treatment), and data was evaluated using one-way ANOVA to determine if measurements were statistically different (p=0.10), and when they were, a post hoc multiple comparison test using LSD was performed. See also Table 2 in Sleighter et al., 2023.

Table 1: Summary of weather conditions during the wheat growing season.

Month	Average Temperature (°C)		Total Rainfall
	Min.	Max.	mm
May	6.7	19.1	32.6
June	5.3	14.3	44.6
July	2.9	13.5	35.8
August	1.3	15.8	9.8
September	3.9	19.8	15.2
October	6.0	25.5	4.8
November	8.4	27.4	16.0
Wheat sown on 5/24/14			
Transit treatment on 8/10/14, at tillering			
Frost events: 8/2-8/4, 8/11-8/13			
Harvest on 11/29/14			

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Table 2: Average sap Brix and yield data from the wheat trial (values below averages in parentheses are 1 standard deviation), where sap Brix was assessed at 1-52 days after treatment.

Treatment	Brix (DAA- days after application)								Yield	
	1 DAA		17 DAA		39 DAA		52 DAA			
Grower Standard (GS)	11.8 (0.8)	b	9.7 (0.6)	b	8.3 (1.2)	b	9.7 (0.6)	b	1052 (60)	b
GS + Transit	14.0 (0.0)	a	13.2 (0.3)	a	12.7 (0.6)	a	13.3 (1.2)	a	1411 (36)	a
% Increase	18.6%		36.1%		53.0%		37.1%		34.1%	
ANOVA, p =	0.0080		0.0007		0.0044		0.0079		0.0009	

- Lentils were grown in Drung (Victoria, Australia) in a heavy clay soil, where the previous crop was wheat, and the lentils were sown into burnt wheat stubble. Sowing conditions were noted as ideal and rainfall for the season was adequate (see Table 3 below). Yields from lentils under cold and frost stress that were treated with Transit alongside the grower standard (GS) fertilizer program were 64% higher than stressed lentils only receiving the GS (without Transit), which was a statistically significant difference (Table 4 below). A considerable frost event occurred about 6 weeks after the treatment and 8 weeks prior to harvest, significantly affecting crop yields in the region. The researcher noted that the increase in yield for the Transit treated plants could be due to the higher sap Brix measurements that were obtained prior to the frost event. Statistically significant increases (54-74%) in sap Brix were observed for the lentils receiving the Transit alongside the GS fertilizer program, which was sustained from 4-31 days after treatment when temperatures were beginning to rise during the Australian spring. This RCBD trial had 4 replicates for the 2 treatments (GS control and GS + Transit treatment), and data was evaluated using one-way ANOVA to determine if measurements were statistically different (p=0.10), and when they were, a post hoc multiple comparison test using LSD was performed. See also Table 2 in Sleighter et al., 2023.

Table 3: Summary of weather conditions during the lentil growing season.

Month	Average Temperature (°C)		Total Rainfall
	Min.	Max.	mm
May	5.6	16.5	60.2
June	2.6	14.7	5.0
July	2.9	14.4	42.0
August	3.4	14.1	57.2
September	4.9	17.5	29.2
October	6.6	22.3	39.4
November	11.3	28.9	49.4
December	11.5	29.2	23.2
Lentils sown on 5/26/17			
Transit treatment on 9/22/17, between V5 and Vn			
Frost event on 11/4/17			
Harvest on 1/2/18			

Table 4: Average sap Brix and yield data from the lentil trial (values below averages in parentheses are 1 standard deviation), where sap Brix was assessed 1 day prior to treatment and for the month following treatment.

Treatment	Brix (DAA- days after application)								Yield	
	-1 DAA		4 DAA		12 DAA		31 DAA			
Grower Standard (GS)	13.0	a	13.3	b	13.5	b	12.8	b	1233	b
	(1.4)		(1.3)		(1.3)		(0.5)		(61)	
GS + Transit	13.3	a	23.0	a	20.8	a	18.8	a	2025	a
	(1.0)		(0.8)		(1.3)		(0.5)		(102)	
% Increase	1.9%		73.6%		53.7%		47.1%		64.3%	
ANOVA, p =	0.78		1.3E-05		0.0002		2.7E-06		1.1E-05	

The results from the 21 trials summarized here and published in the journal Agronomy on corn, soybean, wheat, lentils, sugarcane, tomatoes, peppers, avocados, strawberries that underwent a simulated or naturally-occurring abiotic stress event in the field highlight that stress mitigation due to the addition of Transit is repeatable and reproducible, as plant health improvements were consistently observed.

In addition to the research publication attached to this application, below is a report on 2 field trials done on corn in 2021 and 2022 that highlight the increase in nutrient uptake that occurs with applications of Transit alongside variable nitrogen application rates (175, 200, and 225 lbs/ac nitrogen).

Objectives of the Trials

These independent research studies on corn were conducted in 2021 and 2022 in Whitewater, Wisconsin by Ag Tech Consulting. The objective of these trials was to evaluate the efficacy of Transit 500 on root and shoot biomass weight, yield, nutrient uptake, and fertilizer efficiency. Nutrient use efficiency can be divided into two interactive components: the efficiency of nutrient acquisition (i.e., the amount of nutrient taken up by plants in relation to nutrient supply, which is referred to as nutrient uptake efficiency) and the efficiency of nutrient utilization, which is related to the biomass produced by the nutrient incorporated by plants. Both aspects of nutrient use efficiency were addressed in these trials conducted. Nutrient uptake was measured by harvesting plants within a given area for each treatment, drying the entire sample, weighing it to determine the total biomass, and then measuring the nutrient content in each sample. This allows for a calculation of the total uptake of nutrients for each treatment at a specified time of the plant's development.

Design of the Trials

- **Field Trials**: Treatment plot size: 10' x 50' (500 ft^2 = 0.0115 acres = 46 m^2)
- **Randomized Complete Block Design**, 6 treatments
  - 4 replicates of each treatment in 2021
  - 6 replicates of each treatment in 2022
- **Plant Population**: 35,000 seeds/ac
  - Hybrid DKC 57-75 in 2021, planted at 2.5"
  - Hybrid DKC 101-33 in 2022, planted at 2.25"
- **Previous crop**: soybean
- **Soil Type**: Milford silty clay loam
  - 2021 Testing: pH = 6.9, Cation Exchange Capacity = 26.3, Organic Matter = 4.6%, P = 44 ppm, K = 136 ppm, Ca = 4015 ppm, Mg = 811 ppm, S = 3.1 ppm, B = 0.6 ppm, Mn = 31 ppm, Zn = 3.5 ppm, 11% sand, 66% silt, 23% clay
  - 2022 Testing: pH = 7.2, Cation Exchange Capacity = 26.1, Organic Matter = 5.3%, P = 35 ppm, K = 141 ppm, Ca = 5142 ppm, Mg = 886 ppm, S = 3.4 ppm, B = 0.5 ppm, Mn = 30 ppm, Zn = 3.3 ppm, 20% sand, 50% silt, 30% clay
- **Tillage Operations**: Fall chisel plowed, spring field cultivate to incorporate fertilizer and level field for planting
- **Irrigation**: None
- **Climatic Information**: See appendix
- **Plant evaluation**: Plant vigor, plant and root biomass weights (at V6 in 2021, at V6 and VT in 2022), plant tissues nutrient analysis (at V6 and VT), yield

- **Statistical analysis**: One-way analysis of variance (ANOVA) was utilized to determine if measurements from the replicates across the 6 treatments were statistically different ($p = 0.15$). If $p < 0.15$ from ANOVA, then a post hoc multiple comparison test was performed using least significant difference (LSD) to ascertain which averages were different. Under the guidance of ISO/TC 134, a p value of 0.15 can be used for trials under field conditions.

Fertilizer Protocols and Treatments

- 90 lbs. of 11-52-0 and 200 lbs. of 0-0-62 applied at the end of the previous season
  - On 12/20/2020 for the 2021 trial
  - On 12/1/2021 for the 2022 trial
- 380 lbs. (175 lbs. N), 435 lbs. (200 lbs. N), or 489 lbs. (225 lbs. N) of 46-0-0 applied pre-plant
- 5 gal/ac of 10-34-0 in furrow at planting
- Transit 500 applied in furrow at planting
  - 2.0 oz/ac in 2021 (equivalent to 10 oz/ac of Transit 100 or Transit Soil)
  - 1.6 oz/ac in 2022 (equivalent to 8 oz/ac of Transit 100 or Transit Soil)

Table 5: Treatments for the corn field trials done in 2021 and 2022 in WI.

Treatment	Description
T1	175 lbs/ac N
T2	200 lbs/ac N
T3	225 lbs/ac N
T4	T1 + Transit
T5	T2 + Transit
T6	T3 + Transit

Results of the 2021 Trial

This field trial on corn shows that the addition of Transit to various rates of nitrogen fertilizer provided statistically significant ($p<0.15$) increases in plant vigor, nitrogen in the plant tissue (measured at V6 and VT), plant and root biomass (at V6), and yields at harvest. As shown in Table 6 and Figure 1, the addition of Transit to 175 lb/ac of N provided statistically significant yield increases of 12.6 and 10.1 bu/ac over 175 and 200 lb/ac of N alone, respectively, as well as a numerical increase of 2 bu/ac over 225 lb/ac of N alone. For the 3 rates of N applied, Transit added to the N fertilizer increased yields to a statistically significant degree for each N rate by 7.2% on average, or by 14 bu/ac. Root biomass was also significantly increased for each of the 3 rates of N applied when Transit was included in the application, and shoot biomass was significantly increased at the highest rate of N applied when Transit was included (Table 6).

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Table 6: Data for varying rates of N applied alone and with Transit during the 2021 corn trial.

Treatment	Yield (bu/ac)	Plant Vigor (1-5)	Plant Tissue (%N)		Plant	Root
			V6	VT	V6 Biomass Dry Weight (g)	
T1: 175 lbs/ac N	189.0 c	3.00 c	3.12 c	2.97 d	64.28 b	61.21 d
T2: 200 lbs/ac N	191.5 c	3.00 c	3.19 b	3.09 c	64.75 b	62.56 cd
T3: 225 lbs/ac N	199.6 b	3.25 bc	3.20 b	3.14 bc	65.73 b	64.24 bc
T4: T1 + Transit	201.6 b	3.25 bc	3.18 b	3.16 b	64.98 b	64.17 bc
T5: T2 + Transit	205.6 b	3.50 ab	3.26 a	3.16 b	65.42 b	65.87 b
T6: T3 + Transit	214.9 a	3.75 a	3.27 a	3.26 a	69.56 a	67.90 a

Means followed by the same letter do not significantly differ (p=0.15, LSD).

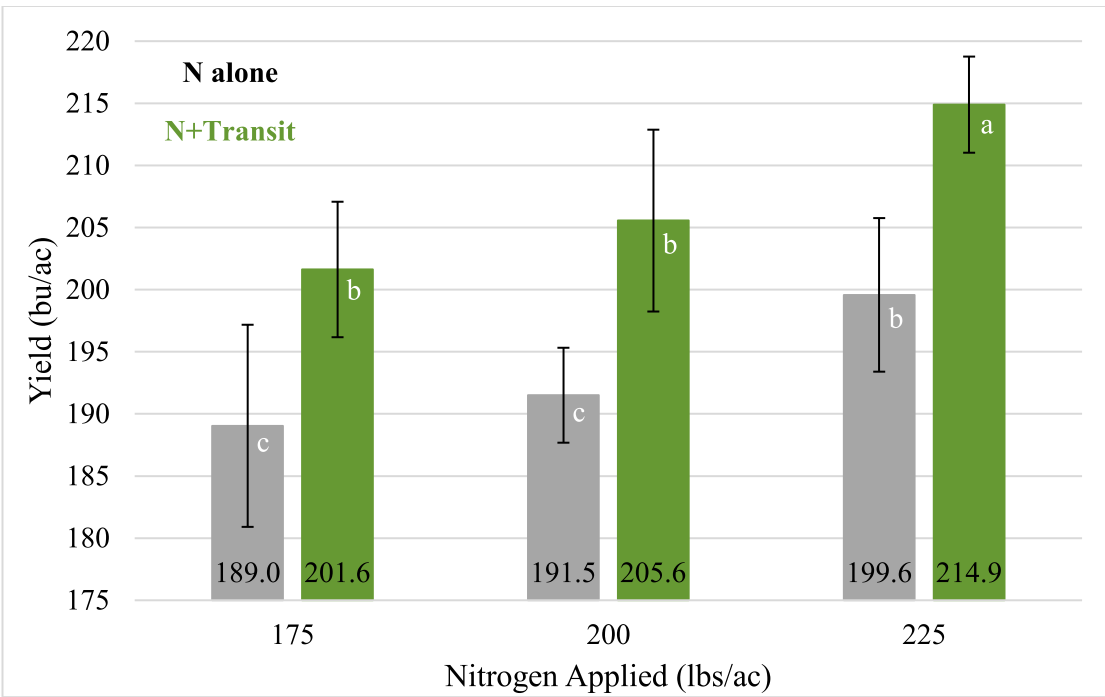

Figure 1: Corn yield in response to varying rates of N applied alone (grey) and with Transit (green) during the 2021 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD). Error bars represent one standard deviation.

Figure 2 shows the plant tissue analysis for %N at V6 and VT. For the 3 rates of N applied, Transit added to the N fertilizer increased %N in the plant tissue by 2.1% and 4.2% on average for the analyses done at V6 and VT, respectively. These increases in %N measured in the plant tissue, with the addition of Transit in the application, were statistically significant at each rate of N applied.

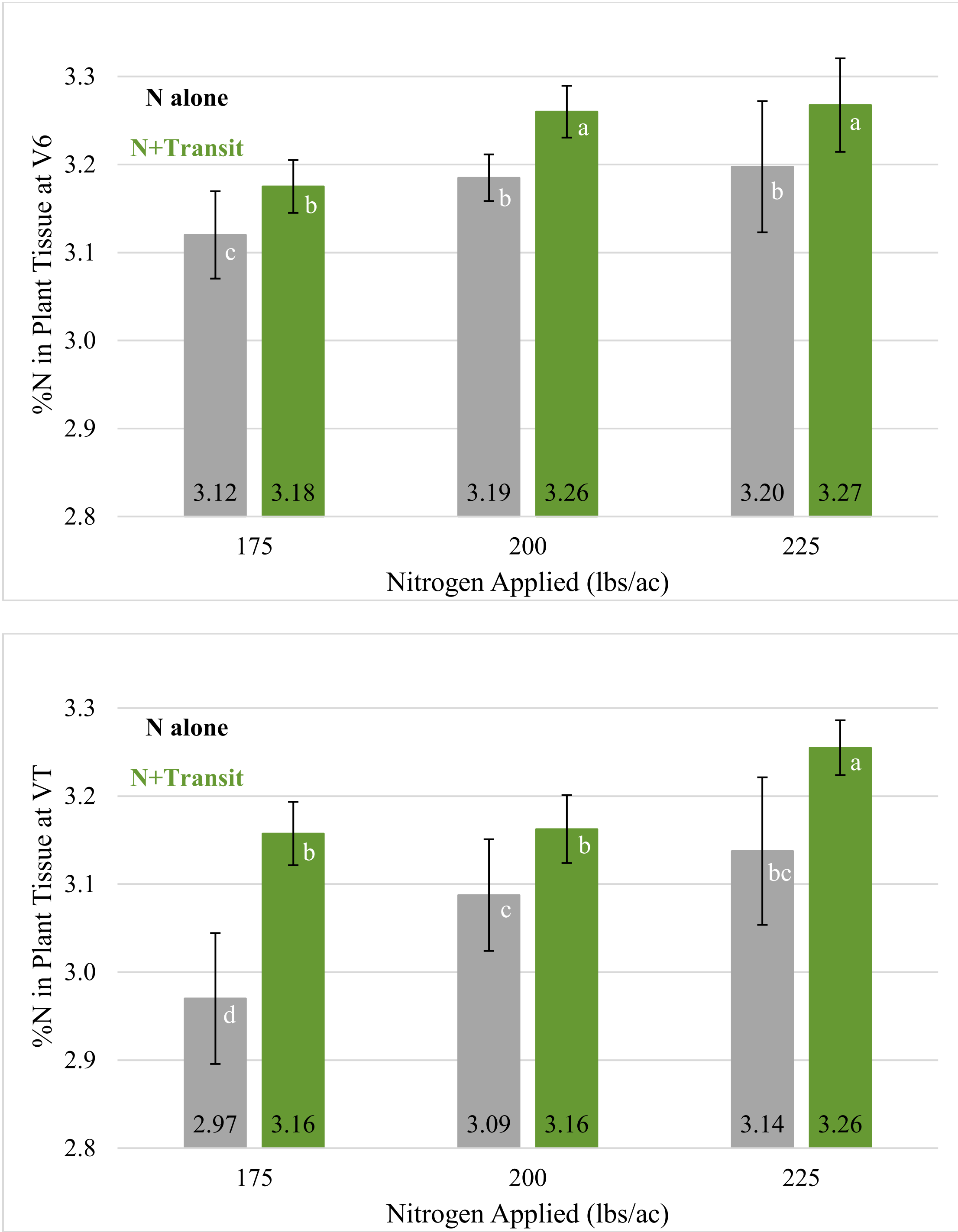

Figure 2: Plant tissue %N at V6 (top) and VT (bottom) in response to varying rates of N applied alone (grey) and with Transit (green) during the 2021 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD). Error bars represent one standard deviation.

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It is highly desirable agronomically and environmentally to be able to lower the pounds of N required to produce a bushel of corn and maintain a higher yield level, and Figure 3 shows the nitrogen use efficiency by calculating the N applied normalized to the yield (lbs N per bushel yield). With the addition of Transit, the lbs of N required to produce a bushel of corn decreases, which was statistically significant at each rate of N applied.

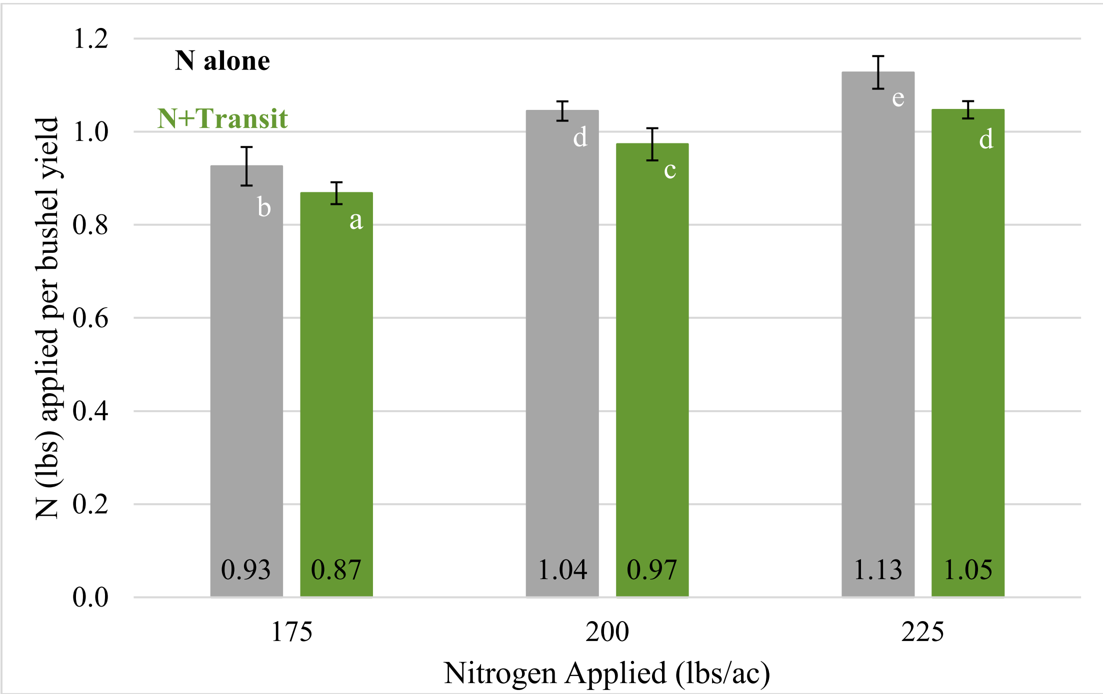

Figure 3: Nitrogen applied per bushel of corn yield for the varying rates of N applied alone (grey) and with Transit (green) during the 2021 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD). Error bars represent one standard deviation.

When the plants were at V6, whole plant samples were collected and analyzed for N, P, K, Ca, Mg, S, B, Mn, Cu, Fe, and Zn, (Table 7), and plant and root biomass measurements were also taken to determine the nutrient uptake for each element (Table 8). The mass of each nutrient per plant was calculated by multiplying the plant dry weight by the percentage of each nutrient as measured. Nutrient uptake per plant was compared for the treatments of N applied alone and with Transit, for each of the 3 N application rates. Nutrient uptake was generally increased with the inclusion of Transit (by 1.2-5.4% on average for all treatments and all nutrients assessed, Figure 4), and interestingly, nutrient uptake was generally increased the most at the highest N rate, by 4.6-11% for all nutrients assessed (Table 8). For the varying rates of N applied, statistically significant increases in nutrient uptake were primarily observed for N, P, Ca, and Mn (Table 8).

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Table 7: Results of the tissue analyses done at V6 for varying rates of N applied alone and with Transit during the 2021 corn trial.

Treatment	Tissue Analysis at V6 (%)										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N	3.12	0.35	2.15	0.41	0.62	0.22	0.0010	0.0032	0.0040	0.011	0.0011
175 + Transit	3.18	0.34	2.15	0.41	0.64	0.22	0.0010	0.0031	0.0042	0.012	0.0010
200 lbs/ac N	3.19	0.34	2.14	0.40	0.64	0.23	0.0012	0.0031	0.0040	0.012	0.0011
200 + Transit	3.26	0.36	2.15	0.41	0.62	0.23	0.0010	0.0032	0.0041	0.012	0.0011
225 lbs/ac N	3.20	0.35	2.15	0.41	0.63	0.22	0.0011	0.0031	0.0042	0.012	0.0010
225 + Transit	3.27	0.35	2.13	0.41	0.64	0.23	0.0011	0.0032	0.0042	0.013	0.0011

Table 8: Calculations of nutrient uptake based on tissue analysis and plant dry masses done at V6 for varying rates of N applied alone and with Transit during the 2021 corn trial. See also Figure 4. Means followed by the same letter do not significantly differ (p=0.15, LSD).

Treatment	Average Nutrient Uptake per Plant (mg)										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N	2005 c	222 cd	1379	260	400 c	138	0.63	2.02	2.59 d	7.36	0.69
175 + Transit	2063 bc	223 cd	1397	263	413 bc	145	0.67	2.00	2.75 bc	7.78	0.65
200 lbs/ac N	2062 bc	219 d	1386	257	413 bc	146	0.74	2.01	2.61 cd	7.54	0.68
200 + Transit	2133 b	235 bc	1408	268	419 b	149	0.65	2.11	2.65 cd	7.62	0.69
225 lbs/ac N	2102 b	230 bc	1410	269	412 bc	146	0.69	2.05	2.76 bc	8.18	0.67
225 + Transit	2273 a	243 a	1482	285	447 a	160	0.77	2.19	2.89 a	8.73	0.73

Treatment	Percent Difference in Nutrient Uptake at V6 with Transit Inclusion										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N + Transit	2.9%	0.4%	1.3%	1.1%	3.1%	4.6%	6.3%	-1.3%	6.1% *	5.7%	-6.0%
200 lbs/ac N + Transit	3.4%	7.8% *	1.6%	4.2%	1.5%	2.2%	-12%	5.1%	1.7%	1.0%	1.0%
225 lbs/ac N + Transit	8.1% *	5.8% *	5.1%	5.8%	8.4% *	9.4%	11%	6.7%	4.6% *	6.7%	8.4%
Average Difference	4.8%	4.7%	2.7%	3.7%	4.3%	5.4%	1.7%	3.5%	4.1%	4.5%	1.2%

* Denotes a statistically significant increase for the rate of N applied.

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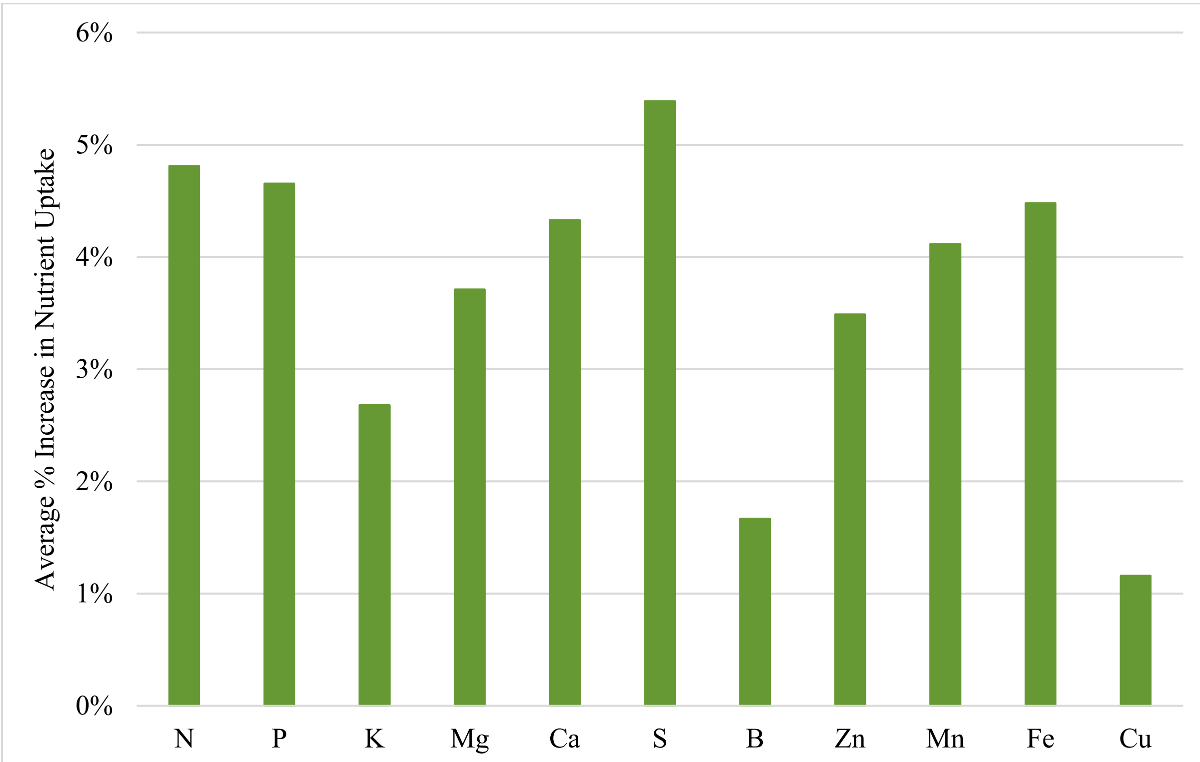

Figure 4: Average percent increase in nutrient uptake for all 3 nitrogen application rates, as measured at V6, when Transit was included in the application during the 2021 corn trial. See also Table 8.

Results of the 2022 Trial

The 2022 trial is a repeat of what was done in 2021 using the same protocol and cooperator (except in the 2022 trial there were 6 replicates per treatment and in 2021 there were 4). This field trial highlights that when decreasing the amount of N applied (from 225 lb/ac N to 200 and 175 lb/ac N) alongside the addition of Transit leads to improved nutrient uptake and sustained (and sometimes larger) yields. Consistent with the 2021 trial, numerical increases (and sometimes statistically significant increases) in plant vigor, nitrogen in the plant tissue, and plant and root biomass were also observed when Transit was included in the application of N.

As shown in Table 9 and Figure 5, for the 3 rates of N applied, Transit added to the N fertilizer increased yields by 1% on average, or by 2 bu/ac (these increases were not statistically significant). In 2021, the improved yields were statistically significant, having an average increase of 7% (or by 14 bu/ac). In the 2021 trial, the yields for the N applications alone at 175, 200, and 225 lb/ac were 189, 192, and 200 bu/ac, respectively. These values are all higher than the US 2021 average reported by the USDA of 177 bu/ac. In 2022, the US average dropped slightly to 173 bu/ac on average. However, in the 2022 trial, the yields for the N applications alone were even higher than in 2021, giving values of 229, 236, and 240 bu/ac for 175, 200, and 225 lb/ac N, respectively. The previous crop on this field was soybeans, and it is likely that there was a significant amount of residual N in the soil and that the lowest N application rate (including the applications made pre-season and at planting) provided sufficient N for the plants. In addition, these very high yields for the control plots highlight the lack of stress that occurred in the field, which can lead to smaller yield and biomass increases due to the presence of a biostimulant.

Table 9: Data for varying rates of N applied alone and with Transit during the 2022 corn trial.

Treatment	Yield (bu/ac)	Plant Vigor (1-5)	Plant Tissue (%N)		Plant (V6)	Root (V6)	Plant (VT)	Root (VT)
			V6	VT	Biomass Dry Weight (g)			
T1: 175 lbs/ac N	228.7 d	3.0 c	3.27 d	3.16 c	65.9 b	63.1 c	201.9 c	190.0 d
T2: 200 lbs/ac N	235.8 bc	3.2 bc	3.36 bc	3.30 b	69.1 b	64.9 c	210.9 ab	196.8 bc
T3: 225 lbs/ac N	240.4 ab	3.7 a	3.39 b	3.39 a	70.6 a	66.9 ab	215.8 a	202.9 ab
T4: T1 + Transit	232.1 cd	3.2 bc	3.32 cd	3.19 c	66.9 b	63.4 c	207.2 bc	190.1 d
T5: T2 + Transit	236.2 bc	3.5 ab	3.36 bc	3.34 b	69.5 b	65.0 bc	210.6 ab	195.5 cd
T6: T3 + Transit	243.3 a	3.7 a	3.45 a	3.40 a	70.2 a	67.6 a	218.0 a	203.8 a

Means followed by the same letter do not significantly differ (p=0.15, LSD).

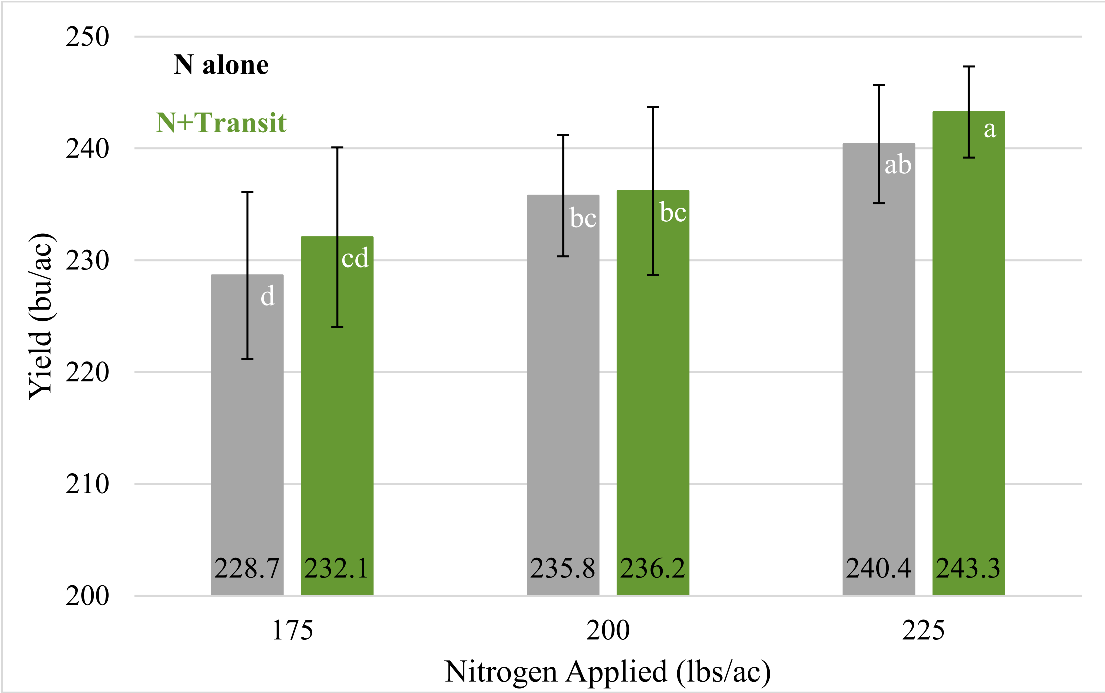

Figure 5: Corn yield in response to varying rates of N applied alone (grey) and with Transit (green) during the 2022 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD). Error bars represent one standard deviation.

Figure 6 shows the plant tissue analysis for %N at V6 and VT. For the 3 rates of N applied, Transit added to the N fertilizer increased %N in the plant tissue by about 1% at each assessment point. While the values obtained for the treatments including Transit in the application were numerically higher, these increases in %N measured in the plant tissue were not statistically significant at p = 0.15, except for the V6 measurements at the highest N application rate.

The ability of Transit to improve the efficiency of N has numerous benefits, not only to the plant but also to the environment as a whole. Figure 7 shows the nitrogen use efficiency by calculating the N applied normalized to the yield (lbs N per bushel yield), highlighting again that less N is required per bushel of yield when Transit is applied alongside nutrition.

When the plants were at both V6 and VT, whole plant samples were collected and analyzed for N, P, K, Ca, Mg, S, B, Mn, Cu, Fe, and Zn, (Table 10), and plant and root biomass measurements were also taken to determine the nutrient uptake for each element (Table 11). Again, the mass of each nutrient per plant was calculated by multiplying the plant dry weight by the percentage of each nutrient as measured. Nutrient uptake per plant was compared for the treatments of N applied alone and with Transit, for each of the 3 N application rates at both V6 and VT. Nutrient uptake was generally increased with the inclusion of Transit by 0.7-6.5% and 0.9-3.3% at V6 and VT, respectively, on average for all treatments and all nutrients assessed (Table 11, Figure 8). For the varying rates of N applied, statistically significant increases in nutrient

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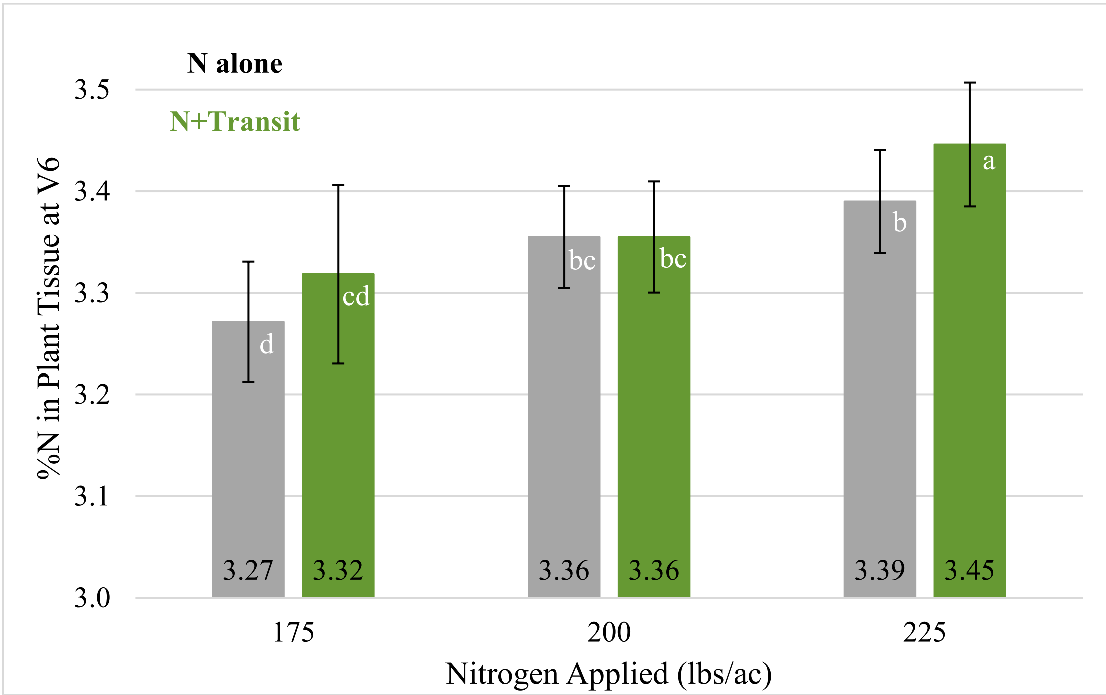
uptake were primarily observed for N, K, Mg, Ca, and Fe for the measurements taken at V6 and for N, K, and Ca for the measurements taken at VT (Table 11).



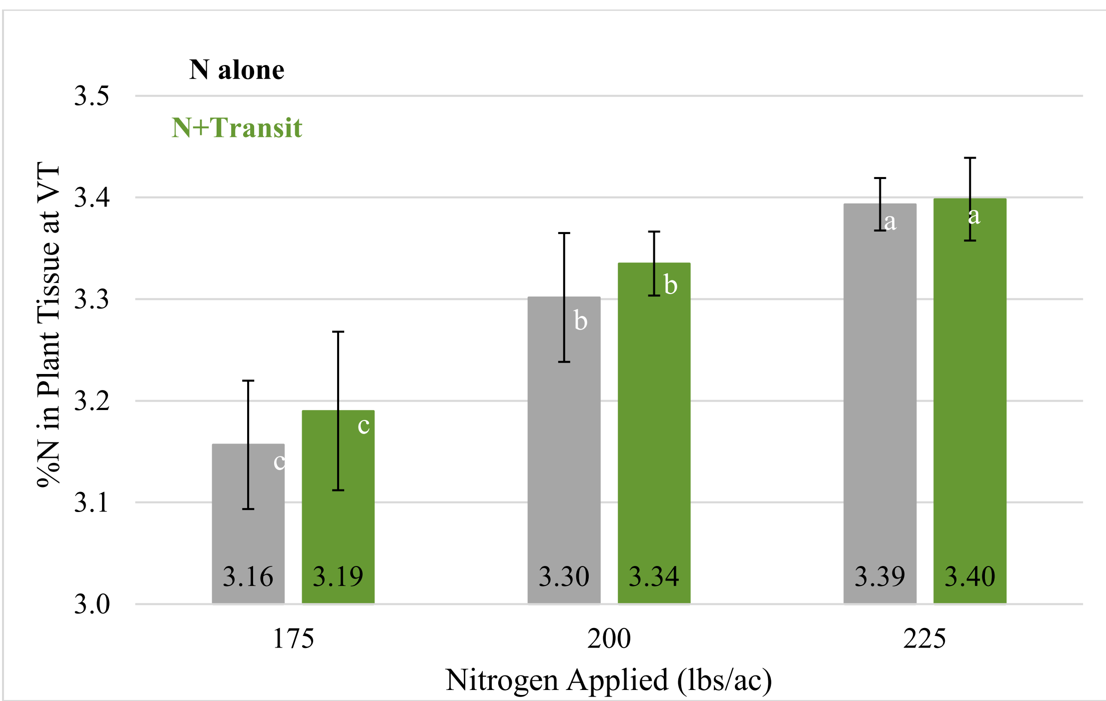


Figure 6: Plant tissue %N at V6 (top) and VT (bottom) in response to varying rates of N applied alone (grey) and with Transit (green) during the 2022 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD). Error bars represent one standard deviation.

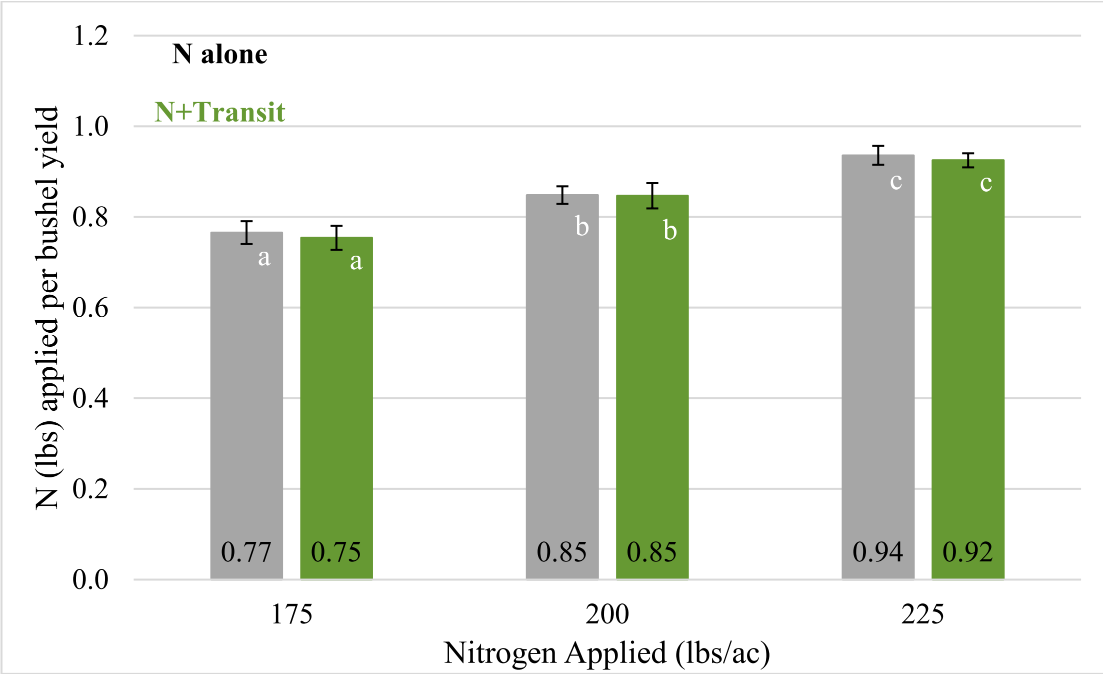


Figure 7: Nitrogen applied per bushel of corn yield for the varying rates of N applied alone (grey) and with Transit (green) during the 2022 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD). Error bars represent one standard deviation.

Table 10: Results of the tissue analyses done at V6 and VT for varying rates of N applied alone and with Transit during the 2022 corn trial.

Treatment	Tissue Analysis at V6 (%)										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N	3.27	0.34	2.15	0.42	0.63	0.23	0.0011	0.0032	0.0042	0.012	0.0010
175 + Transit	3.32	0.34	2.16	0.44	0.63	0.24	0.0011	0.0033	0.0043	0.013	0.0010
200 lbs/ac N	3.36	0.34	2.16	0.43	0.64	0.23	0.0011	0.0033	0.0042	0.012	0.0010
200 + Transit	3.36	0.35	2.16	0.43	0.64	0.24	0.0011	0.0034	0.0043	0.013	0.0011
225 lbs/ac N	3.39	0.35	2.16	0.43	0.63	0.24	0.0012	0.0033	0.0042	0.013	0.0010
225 + Transit	3.45	0.35	2.15	0.44	0.64	0.24	0.0013	0.0033	0.0042	0.013	0.0012

Treatment	Tissue Analysis at VT (%)										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N	3.16	0.42	2.24	0.42	0.63	0.24	0.0011	0.0033	0.0024	0.014	0.0011
175 + Transit	3.19	0.42	2.25	0.43	0.63	0.24	0.0011	0.0033	0.0025	0.014	0.0011
200 lbs/ac N	3.30	0.42	2.25	0.42	0.63	0.24	0.0011	0.0034	0.0026	0.014	0.0011
200 + Transit	3.34	0.42	2.25	0.43	0.64	0.24	0.0011	0.0034	0.0025	0.014	0.0011
225 lbs/ac N	3.39	0.44	2.26	0.43	0.64	0.24	0.0011	0.0034	0.0025	0.014	0.0011
225 + Transit	3.40	0.44	2.25	0.43	0.64	0.24	0.0011	0.0034	0.0026	0.014	0.0011

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Table 11: Calculations of nutrient uptake based on tissue analysis and plant dry masses done at V6 and VT for varying rates of N applied alone and with Transit during the 2022 corn trial. Means followed by the same letter do not significantly differ (p=0.15, LSD).

Treatment	Average Nutrient Uptake per Plant (mg) at V6										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N	2155 d	223 d	1413 b	276 d	413 b	153	0.74	2.11	2.74	8.20 c	0.65
175 + Transit	2219 c	230 cd	1441 b	292 c	419 b	159	0.76	2.23	2.84	8.89 ab	0.66
200 lbs/ac N	2317 b	236 bc	1490 a	298 bc	441 a	159	0.73	2.24	2.90	8.59 bc	0.71
200 + Transit	2332 ab	243 ab	1500 a	295 bc	444 a	163	0.79	2.33	3.00	9.37 a	0.74
225 lbs/ac N	2393 ab	249 a	1522 a	306 ab	447 a	166	0.84	2.33	2.98	9.15 ab	0.72
225 + Transit	2420 a	243 ab	1513 a	310 a	448 a	169	0.89	2.29	2.97	8.97 ab	0.82

Treatment	Percent Difference in Nutrient Uptake at V6 with Transit Inclusion										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N + Transit	3.0% *	3.0%	2.0%	6.0% *	1.5%	4.4%	3.0%	5.7%	3.5%	8.4% *	1.5%
200 lbs/ac N + Transit	0.6%	3.1%	0.7%	-0.9%	0.6%	2.8%	8.6%	3.7%	3.4%	9.1% *	3.9%
225 lbs/ac N + Transit	1.2%	-2.4%	-0.6%	1.4%	0.3%	1.6%	6.5%	-1.5%	-0.1%	-2.0%	14%
Average Difference	1.6%	1.2%	0.7%	2.2%	0.8%	3.0%	6.1%	2.7%	2.3%	5.2%	6.5%

Treatment	Average Nutrient Uptake per Plant (mg) at VT										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N	6373 d	855 b	4529 d	856	1262 d	474	2.15	6.66	4.91	27.49	2.22
175 + Transit	6611 c	870 b	4660 c	891	1302 c	504	2.35	6.91	5.25	28.29	2.25
200 lbs/ac N	6962 b	893 b	4741 b	893	1332 bc	499	2.35	7.10	5.41	28.82	2.25
200 + Transit	7024 b	888 b	4728 b	913	1337 bc	502	2.32	7.13	5.30	28.89	2.35
225 lbs/ac N	7324 a	939 a	4871 a	935	1371 ab	514	2.37	7.23	5.47	29.68	2.34
225 + Transit	7408 a	952 a	4905 a	934	1391 a	520	2.43	7.34	5.63	29.57	2.43

Treatment	Percent Difference in Nutrient Uptake at VT with Transit Inclusion										
	N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
175 lbs/ac N + Transit	3.7% *	1.8%	2.9% *	4.1%	3.2% *	6.3%	9.1%	3.7%	6.9%	2.9%	1.1%
200 lbs/ac N + Transit	0.9%	-0.5%	-0.3%	2.2%	0.4%	0.6%	-1.6%	0.4%	-2.1%	0.2%	4.6%
225 lbs/ac N + Transit	1.1%	1.4%	0.7%	-0.2%	1.5%	1.0%	2.5%	1.5%	3.0%	-0.3%	4.1%
Average Difference	1.9%	0.9%	1.1%	2.1%	1.7%	2.6%	3.3%	1.9%	2.6%	0.9%	3.3%

* Denotes a statistically significant increase for the rate of N applied.

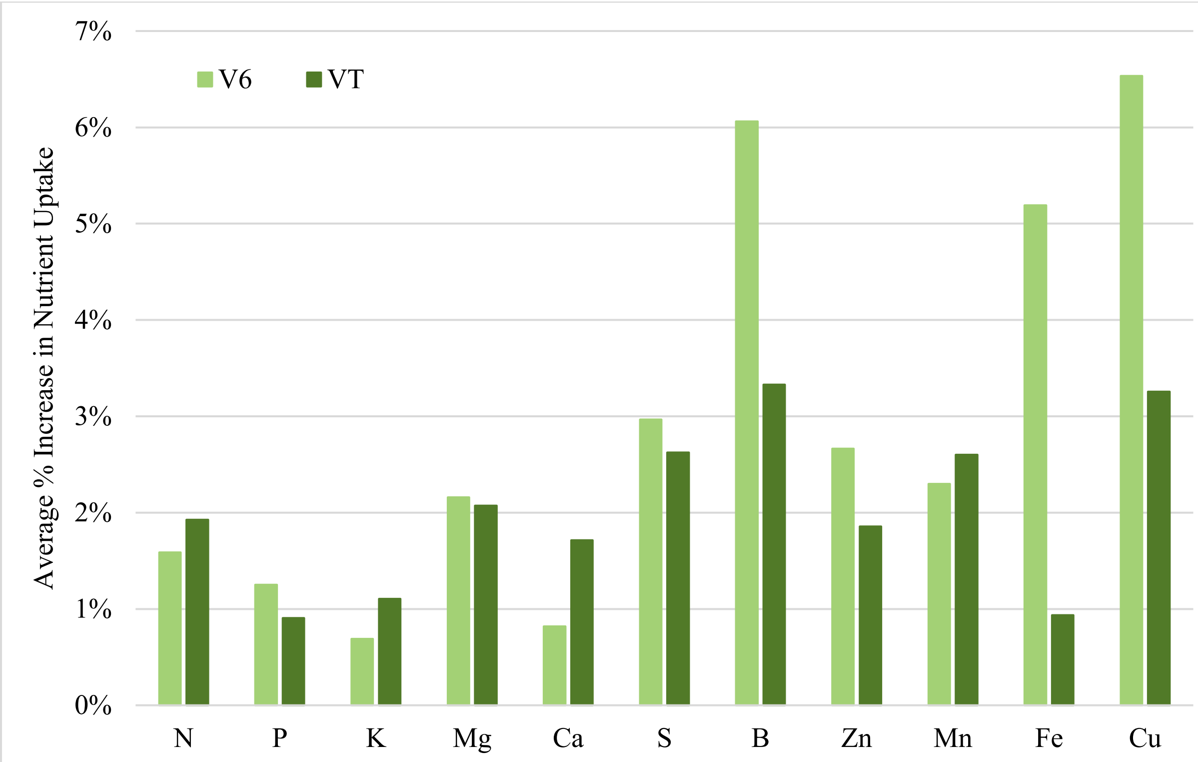

Figure 8: Average percent increase in nutrient uptake for all 3 nitrogen application rates, as measured at V6 and VT, when Transit was included in the application during the 2022 corn trial. See also Table 11.

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Conclusions

The field trials on corn in 2021 and 2022 show that the addition of Transit to various rates of nitrogen fertilizer provided statistically significant increases in plant vigor, fertilizer efficiency, nitrogen and other nutrient uptake, plant and root biomass, and yields at harvest. The average yield increase at 175, 200, and 225 lbs/ac N with the addition of Transit was 7% in 2021 and 1% in 2022, giving a 2-year average of 4% (across all 3 rates of N application). Nutrients measured in the plant tissue, alongside measurements of plant dry weight, at V6 in 2021 and at V6 and VT in 2022 indicate increases in nutrient uptake when Transit is applied. The average increase in uptake due to the application of Transit for all nutrients at all assessments was 2.9%. With the inclusion of Transit into the fertilizer application, sustained yields at lower N rates can still be achieved, as the lbs of N required to produce a bushel of corn decreases. In the 2021 trial, the inclusion of Transit with 175 lb/ac N had a yield exceeding the groups receiving 200 or 225 lbs/ac N applied alone. In the 2022 trial, where even the control plots had very high yields at harvest (all >225 bu/ac), the treatment including Transit with 175 lb/ac N had a yield that was statistically equivalent to that of the treatment at 200 lbs/ac N alone. Likewise in 2022, the treatment including Transit with 200 lb/ac N had a yield that was statistically equivalent to that of the treatment at 225 lbs/ac N alone. Overall, a single biostimulant application in-furrow alongside the grower standard fertilizer program at planting provided increases in nutrient uptake by the plant and yields that were significantly higher than the control or yields that were sustained even when reducing the N application by 25 lb/ac.

The research article published in Agronomy in 2023 for the 21 trials on corn, wheat, soybean, sugarcane, tomatoes, peppers, strawberries, avocados, and lentils highlights that Transit improves the tolerance to and recovery from abiotic stress, including heat, drought, cold/frost, excess moisture, and salinity. In addition to abiotic stress mitigation, the manuscript also provides data to corroborate the claims of increases in sap Brix and enhancement of fertilizer efficiencies, which aid the plant's ability to improve root and shoot growth and increase biomass.

The data from the summation of 23 trials discussed here (between the research publication and efficacy report on the 2021 and 2022 corn field trials in Wisconsin) together document the efficacy for Transit to support the following list of claims:

- Improves tolerance to and recovery from abiotic stress
- Increases sap Brix
- Enhances fertilizer efficiencies
- Increases nutrient uptake
- Aids the plant's ability to improve root and shoot growth and increase biomass

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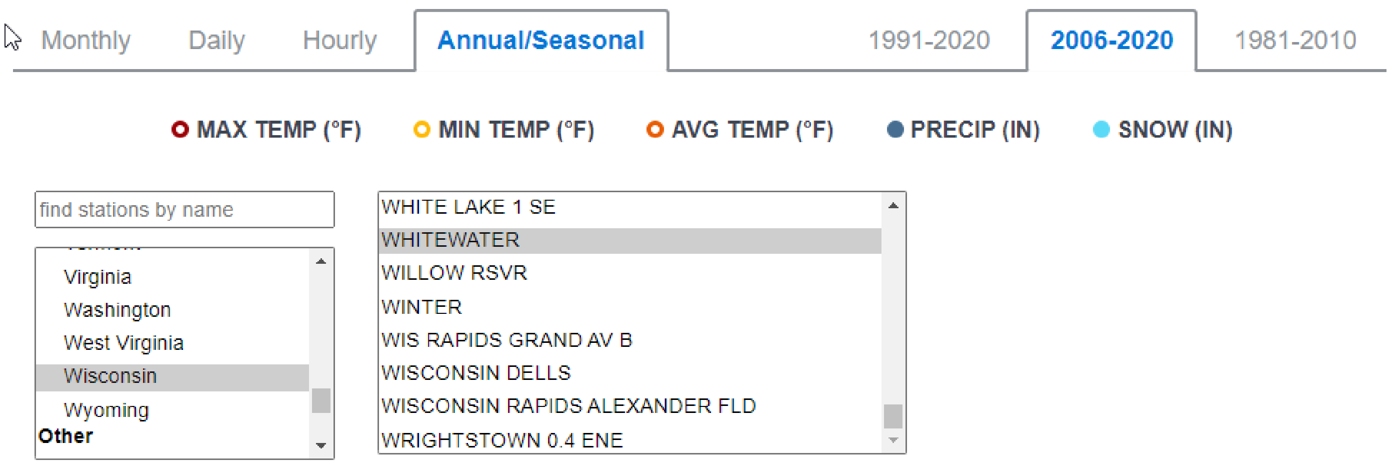
Appendix: Climatic Information

Climatic information for Whitewater, WI was found using the National Oceanic and Atmospheric Administration (NOAA) U.S. Climate Normals Quick Access page (https://www.ncei.noaa.gov/access/us-climate-normals/#dataset=normals-annualseasonal&timeframe=15).



WHITEWATER, WI

Get this data as .csv | .pdf
Station info: USC00479190

Season	MAX TEMP (°F)	MIN TEMP (°F)	AVG TEMP (°F)	PRECIP (IN)
Annual	56.6	37.3	47.0	39.49
Winter	30.1	14.3	22.2	5.68
Spring	56.3	35.9	46.1	10.27
Summer	80.3	59.1	69.7	14.08
Autumn	59.9	39.8	49.9	9.46