

2020 Hemp Flower Nitrogen Fertility Trial



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Hemp is a non-psychoactive variety of *Cannabis sativa* L. The crop is one of historical importance in the U.S. and re-emerging worldwide importance as medical providers and manufacturers seek hemp as a renewable and sustainable resource for a wide variety of consumer and industrial products. Hemp grown for all types of end-use (health supplement, fiber, and seed) contains less than 0.3% tetrahydrocannabinol (THC). Some hemp varieties intended to produce a health supplement contain relatively high concentrations of a compound called cannabidiol (CBD), potentially 10-15%. The compound CBD has purported benefits such as relief from inflammation, pain, anxiety, seizures, spasms, and other conditions. The CBD compound is the most concentrated in the female flower buds of the plant, however, it is also in the leaves and other plant parts as well.

To produce hemp for flower, the plant is generally grown intensively as a specialty crop and the flowers are cultivated for maximum growth. The various cannabinoids and terpenes concentrated in the flower buds are often extracted and incorporated into topical products (salves, lip balm, lotion) and food and is available in pill capsules, powder form, and more, which can be found in the market today. To help farmers succeed, agronomic research on hemp is needed in the United States. University of Vermont in partnership with <u>CASE Institute (https://www.caseinstitute.org/)</u>, evaluated the impact of five different nitrogen (N) application rates on the growth habit, yield, flower quality, and whole plant nutrient concentration of hemp.

MATERIALS AND METHODS

The trial was initiated at Borderview Research Farm in Alburgh, Vermont (Table 1) and the experimental design was a randomized complete block design with four replications. Plots consisted of five plants spaced 5' apart in the row and plot treatments consisted of five N application rates including a Control (0 lbs N ac⁻¹), 75, 100, 125, and 150 lbs N ac⁻¹.

Location	Borderview Research Farm
	Alburgh, VT
Soil type	Benson rocky silt loam, 3-5% slope
Previous crop	Winter Canola
Plot size	5' x 20'
Plant spacing (ft)	5' x 5'
Plant material	Seedling
Planting date	9-Jun
Harvest date	8-Oct

Table 1. Agronomic information for the hemp variety trial, Alburgh, VT, 2020.

The 4-week old hemp seedlings (variety Lifter) were transplanted on 9-Jun into a seed bed prepared with conventional tillage. A cover crop mixture of crimson clover and annual ryegrass was planted between rows on 15-Jun. Drip irrigation was setup to supply moisture as needed by the hemp plants. Plots received

nitrogen fertility in split applications over an eight-week period starting on 26-Jun in the form of ammonium nitrate plus sulfur (URAN 28-0-0) applied directly to individual plants (Table 2).

Treatment	Total application rate 28-0-0	Weekly application rate	Weekly application rate
lbs N ac ⁻¹	gal ac ⁻¹	gal ac ⁻¹	mL plant ⁻¹
0	0	0	0
75	23.1	2.89	6.27
100	30.8	3.85	8.36
125	38.5	4.81	10.5
150	46.1	5.77	12.5

Irrigation was applied on a weekly basis at a rate of 8000 gallons of water per acre delivered via drip tape. Irrigation duration and amount was modified based on weekly rainfall. Prior to harvest, plant height and width was measured from all harvested plants in each plot. From each plot, flower samples were taken from the top 8" of colas and were sent to Bia Diagnostics (Colchester, VT) for analysis of major cannabinoids.

For each plant harvested, the whole plant weight was recorded. On 8-Oct, all plants were harvested and were broken down into smaller branched sections and larger "fan" or "sun" leaves were removed by hand, while smaller leaves were left attached since they subtend from the flower bract. Remaining stems were then bucked using the BuckmasterPro Bucker (Maple



Image 1. Centurion Pro Gladiator Trimmer (Maple Ridge, BC, Canada).

Ridge, BC, Canada) and remaining leaf material and buds were collected. Wet bud and leaf material was then run through the CenturionPro Gladiator Trimmer (Maple Ridge, BC, Canada) (Image 1).

Wet bud weight and unmarketable bud weight were recorded. The flower buds were then dried at 80° F or ambient temperature with airflow until dry enough for storage without molding. A subsample of flower bud from each plot was dried in a small dehydrator and wet weights and dry weights were recorded in order to calculate the percent moisture of the flower buds. The percent moisture at harvest was used to calculate dry matter yields. Metrics were collected for each of the two harvested plants within each plot and a plot average was calculated.

The day prior to harvest (7-Oct), one plant per plot was harvested and chipped to be analyzed for whole plant nutrient concentrations. A subsample of chipped plants was taken, dried, and sent to Dairy One in Ithaca, NY for nutrient analysis.

Yield data and stand characteristics were analyzed using mixed model analysis using the mixed procedure of SAS (SAS Institute, 1999). Replications within the trial were treated as random effects, and treatments were treated as fixed. Treatment mean comparisons were made using the Least Significant Difference (LSD) procedure when the F-test was considered significant (p<0.10).

Variations in yield and quality can occur because of variations in genetics, soil, weather, and other growing conditions. Statistical analysis makes it possible to determine whether a difference among treatments is real or whether it might have occurred due to other variations in the field. At the bottom of each table a p-value is presented for each variable that showed statistical significance (p-value ≤ 0.10). In this case, the difference between two treatments within a column is equal to or greater than the least significant difference (LSD) value and you can be sure that for 9 out of 10 times, there is a real difference between the two treatment C is significantly different from treatment A but not from treatment B. Treatment B and treatment C have share the same letter 'a' next to their yield value, to indicate that these results are statistically similar. The difference between treatment C and treatment B is equal to 1.5, which

is less than the LSD value of 2.0. This means that these treatments did not differ in yield. The difference between treatment C and treatment A is equal to 3.0, which is greater than the LSD value of 2.0. This means that the yields of these treatments were significantly different from one another. The letter 'b' next to treatment A's yield value shows that this value is significantly different from treatment B and treatment C, which have the letter 'a' next to their value.

Treatment	Yield
А	6.0 b
В	7.5a
С	9.0 a
LSD (p-value ≤ 0.10)	2.0

Participants of State Hemp Programs intending to grow should acknowledge state and federal regulations regarding hemp production and registration. Growers must register within their intended state for production and must adhere to most current or active rules and regulations for production within a grower's given state. Regulations are subject to change from year to year with the development and approval of proposed program rules and it is important to note that regulations may vary across state lines and may be impacted by pending federal regulations. Please refer to the following link for a detailed outline of proposed rules in Vermont, as well as additional information regarding the Vermont Agency of Agriculture, Food and Markets (VAAFM) Hemp Program:

https://agriculture.vermont.gov/public-health-agricultural-resource-management-division/hemp-program.

RESULTS

Seasonal precipitation and temperature were recorded with a Davis Instrument Vantage Pro2 weather station, equipped with a WeatherLink data logger at Borderview Research Farm in Alburgh, VT (Table 3). The growing season was defined by hot and dry conditions throughout the summer months, punctuated by a handful of larger, infrequent rain events seen largely in August. June was especially dry during the transplant and establishment period for our hemp trials with below average precipitation in much of the growing season. Average temperatures during the growing period were 4.11 degrees higher than the 30-year average for the season with a 5.5% higher growing degree day accumulation for the year.

Alburgh, VT	June	July	August	September	October
Average temperature (°F)	66.9	74.8	68.8	59.2	48.3
Departure from normal	1.08	4.17	0.01	-1.33	0.19
Precipitation (inches)	1.86	3.94	6.77	2.75	3.56
Departure from normal	-1.77	-0.28	2.86	-0.91	0.00
Growing Degree Days (Base 50°F)	516	751	584	336	126
Departure from normal	35	121	2	-24	-6

Table 3. Seasonal weather data collected in Alburgh, VT, 2020.

Based on weather data from a Davis Instruments Vantage Pro2 with WeatherLink data logger. Historical averages are for 30 years of NOAA data (1981-2010) from Burlington, VT.

Plant height did not differ significantly between N application rates (Table 4) with plants reaching an average of 157 cm tall. Whole plant weights were significantly different across treatments with plants receiving no supplemental nitrogen (control) and the 75 lbs N ac⁻¹ rates having the lowest average plant weights compared to the top performer of 100 lbs N ac⁻¹ at 16.5 lbs plant⁻¹. The 150 and 125 lbs N ac⁻¹ rates were statistically similar to the top performer, but weights were slightly lower at 15.8 and 15.0 lbs plant⁻¹ respectively.

Treatment	Plant height	Plant weight
lbs N ac ⁻¹	cm	lbs plant ⁻¹
Control	159	14.0 b†
75	152	14.2 b
100	155	16.5 a
125	161	15.0 ab
150	155	15.8 ab
LSD (0.10) ‡	NS¥	2.22
Trial Mean	157	15.1

Table 4. Hemp whole plant weight, height, and width, Alburgh, VT, 2020.

†Within a column treatments marked with the same letter were statistically similar (p=0.10).

‡LSD – Least significant difference at p=0.10.

 \mathbf{Y} NS – No significant difference between treatments.

Total bud weight, leaf weight, and stem weight were measured at harvest to further evaluate growth characteristics of each nitrogen application rate (Table 5). In general, plants across treatments appeared to be fairly uniform in growth habit with little to no observable differences in appearance. Across the trial, very few differences were apparent with only the bud weight of plants showing some treatment effect. Plants receiving the 100 lbs N ac⁻¹ treatment had the highest overall average bud weight at 6.87 lbs plant⁻¹ and were statistically similar to the 150 lbs N ac⁻¹ treatment at 6.64 lbs plant⁻¹. Other treatments yielded approximately 1 lb less per plant. With the Lifter cultivar in this trial, plants were on average 41.3% bud material, 25.2% stem, and 33.5% leaf. While leaf weights were not significantly different across treatments, the highest three rates of nitrogen did have the highest amount of leaf material within the trial, especially when comparing to the leaf weight of the control at 4.77 lbs plant⁻¹. The amount of total leaf or stem material

can influence a number of factors such as harvest time to remove excess leaf material for trimmed flower or harvestable plant material in a biomass production system. Amount of time required to harvest plants could vary drastically depending on desired end-product and intricacy of trimming, influenced largely by overall plant size and proportions of bud, leaf, and stem material.

Treatment	Stem weight	Stem weight	Bud weight	Bud weight	Leaf weight	Leaf weight	Bud:stem	Leaf:stem
lbs N ac-1	lbs plant ⁻¹	% total	lbs plant ⁻¹	% total	lbs plant ⁻¹	% total		
Control	3.40	24.6	5.84 b†	42.1	4.77	33.3	1.74	1.40
75	3.69	25.8	5.62 b	40.6	4.84	33.7	1.60	1.31
100	4.34	26.2	6.87 a	41.8	5.29	32.0	1.60	1.24
125	3.94	25.4	5.68 b	39.8	5.34	34.8	1.64	1.39
150	3.72	23.8	6.64 a	42.4	5.47	33.7	1.79	1.46
LSD (0.10) ‡	NS¥	NS	0.667	NS	NS	NS	NS	NS
Trial Mean	3.82	25.2	6.13	41.3	5.14	33.5	1.67	1.36

Table 5. Hemp plant growth metrics, Alburgh, VT, 2020.

[†]Within a column treatments marked with the same letter were statistically similar (p=0.10).

LSD – Least significant difference at p=0.10.

 \mathbf{Y} NS – No significant difference between treatments.

At harvest, a composite subsample of flower material was collected from each plot and dried down to determine flower dry matter and calculate dry matter flower yields (Table 6). Flower dry matter was not significantly different across treatments. Plants receiving the 100 lbs N ac⁻¹ rate had the highest dry matter yields at 2884 lbs ac⁻¹ alongside the 150 lbs N ac⁻¹ rate at 2877 lbs ac⁻¹. Those rates receiving additional fertility appeared to have the lowest amounts of unmarketable flower with the highest rate having on average 0.012 lbs plant⁻¹ compared to the control which had the highest amount of unmarketable flower material. Unmarketable flower included any flower that had suffered from disease, rot, soil contamination, or otherwise damaged flower material. Dry matter flower yields for the Lifter cultivar within the trial averaged 2629 lbs ac⁻¹ with an average flower dry matter of 24.7%.

Treatment	Flower dry matter	Unmarketable wet flower yield	Dry matter flower yield ϵ
lbs N ac ⁻¹	%	lbs plant ⁻¹	lbs ac ⁻¹
Control	25.4	0.072 b	2586 b†
75	24.4	0.038 ab	2389 b
100	24.1	0.016 ab	2884 a
125	24.4	0.050 ab	2407 b
150	25.0	0.012 a	2877 а
LSD (0.10) ‡	NS¥	0.058	302
Trial Mean	24.7	0.037	2629

Table 6. Hemp flower bud yield, Alburgh, VT, 2020.

*Within a column treatments marked with the same letter were statistically similar (p=0.10).

‡LSD – Least significant difference at p=0.10.

 \mathbf{Y} NS – No significant difference between treatments.

€Dry matter yield is reported at 0% moisture.

Dried flower samples were also analyzed for CBD and THC concentrations and a CBD:THC ratio was calculated (Table 7). Results for cannabinoids are on a dry matter basis (0% moisture). Each of the analyzed cannabinoids, with the exception of D9-THC, showed statistically significant treatment responses to nitrogen fertility rates. For both CBDA and THCA, peak concentrations were observed in the 75 lbs N ac⁻¹ treatment at 18.3% and 0.597% respectively, and was statistically similar to the Control, 100, and 125 lbs N ac⁻¹ treatments. The CBD concentrations were again highest in the 75 lbs N ac⁻¹ at 0.738% alongside similarly high values seen in the control at 0.602%. Highest values for total CBD were observed in the 75 lbs N ac⁻¹ treatments at 16.8% and were statistically similar to the Control and 100 lbs N ac⁻¹ treatments at 14.4% total CBD each. The 150 lbs N ac⁻¹ treatment was consistently the lowest for all tested values for each analyzed cannabinoid and total cannabinoids resulting in a nearly 4% difference in total CBD. While the concentrations appeared to be impacted by nitrogen fertility rates, the ratio of CBD:THC was not impacted, remaining fairly consistent across all treatments. As concentrations of CBD increased or decreased for a given treatment, THC followed similar trends leading to proportionally similar cannabinoid concentrations for those analyzed.

					D9-							CBD :
Treatment	CBI	DA	CBE)	THC	THC	A	Total TH	Сŧ	Total CE	BD ŧ	THC
lbs N ac ⁻¹	%)	%		%	%		%		%		%
Control	15.8	ab†	0.602	ab	0.054	0.522	ab	0.512	ab	14.4	ab	28.2
75	18.3	а	0.738	a	0.071	0.597	а	0.594	a	16.8	а	28.3
100	15.7	ab	0.570	b	0.056	0.509	ab	0.503	ab	14.4	ab	28.5
125	15.2	ab	0.560	b	0.052	0.501	ab	0.492	ab	13.9	b	28.3
150	14.1	b	0.577	b	0.047	0.455	b	0.447	b	13.0	b	29.2
LSD (0.10) ‡	3.16		0.145		NS ¥	0.102		0.110		2.88		NS
Trial mean	15.8		0.610		0.056	0.517		0.509		14.5		28.5

Table 7. Hemp flower concentrations, Alburgh, VI	Τ, 2020.
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[†]Within a column treatments marked with the same letter were statistically similar (p=0.10).

‡LSD – Least significant difference at p=0.10.

¥NS – No significant difference between treatments.

t Total potential CBD = (0.877 x CBDA) + CBD.

 \pm Total potential THC = (0.877 x THCA) + Δ -9 THC

There were significant differences across treatments for concentrations of potassium, phosphorus, calcium, manganese, iron, and boron (Table 8). Highest values for potassium, phosphorus, and calcium were seen in the control at 1.94%, 0.635%, and 2.43% respectively. Potassium and phosphorus concentrations reacted similarly with statistically similar values observed in the 75 and 150 lbs N ac⁻¹ treatments. Lowest values for each of these three nutrients was seen at the 125 lbs N ac⁻¹ treatment. Nitrogen management of soil is closely linked to the plant uptake of a wide number of nutrients. Differences in primary and secondary nutrient uptake could have been impacted by changes in soil pH as a result of increased nitrogen application rates or weather conditions.

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Treatment	Nitrogen	Potassium		Phosphorus		Calcium		Magnesium	Carbon
lbs N ac ⁻¹	%	%		%		%		%	%
Control	2.83	1.94	a†	0.635	а	2.43	а	0.281	17.7
75	2.81	1.79	ab	0.566	ab	2.36	ab	0.290	17.9
100	2.81	1.71	b	0.530	b	2.35	ab	0.273	17.8
125	2.78	1.70	b	0.507	b	2.19	b	0.272	18.1
150	2.74	1.79	ab	0.550	ab	2.26	ab	0.284	18.3
LSD (0.10) ‡	NS	0.17		0.088		0.22		NS ¥	NS
Trial Mean	2.79	1.78		0.557		2.32		0.280	18.0
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Table 8. Hemp whole plant nutrient analysis, Alburgh, VT, 2020.

†Within a column treatments marked with the same letter were statistically similar (p=0.10).

LSD – Least significant difference at p=0.10.

 \mathbf{Y} NS – No significant difference between treatments.

Treatment	Manganese		Iron		Copper	Boron		Zinc
lbs N ac ⁻¹	ppm		ppm		ppm	ppm		ppm
Control	64.8	b†	329	а	9.47	29.7	ab	40.0
75	63.3	b	269	b	8.58	27.3	а	36.4
100	86.3	а	300	ab	8.72	31.2	ab	38.3
125	67.5	b	303	ab	8.04	26.9	b	36.0
150	70.8	b	259	b	9.28	26.4	b	38.9
LSD (0.10) ‡	13.6		53.9		NS ¥	4.03		NS
Trial Mean	70.5		292		8.82	28.3		37.9

[†]Within a column treatments marked with the same letter were statistically similar (p=0.10).

‡LSD – Least significant difference at p=0.10.

 \mathbf{Y} NS – No significant difference between treatments.

DISCUSSION

As we continue to investigate nitrogen response in high cannabinoid hemp, some similarities can be observed between past research done in grain and fiber. Some grain and fiber hemp research have shown that the majority of nitrogen uptake occurs during the first month of growth during vegetative periods. This ends up being a critical growth period for high cannabinoid hemp as well with the rapid uptake of nitrogen occurring during the vegetative production period. Additionally, a positive yield and biomass response in grain and fiber varieties is seen with increased nitrogen application rates from up to approximately 130 lbs N ac⁻¹. Past this point, additional nitrogen appears to have no major impact on growth. Within this trial, those treatments that received the highest three nitrogen application rates resulted in greatest whole plant biomass, showing some similarities to past research results in grain and fiber hemp. Nitrogen rates in this study appeared to have an impact on overall plant weight with 100 lbs N ac⁻¹ rate having the highest plant weight. Comparable plant weights were observed in the 125 and 150 lbs N ac⁻¹ rates with the lowest two treatments also having lowest plant weights overall.

Nitrogen rates within this study also appeared to have some impact on hemp flower dry matter yields for this cultivar, with those treatments receiving 100 and 150 lbs N ac⁻¹ resulting in highest yields. Additionally,

unmarketable flower appeared to be positively impacted by additional nitrogen applications with the highest rate having the lowest amount of unmarketable flower material and the control having the highest amount of unmarketable flower material. With the maturation rate of the selected cultivar for this trial and potentially as a result of disease resistance, there appeared to be little to no observable pest issues in this trial, whereas adjacent trials suffered from powdery mildew issues as well as high populations of aphids later in the season.

Concentrations of analyzed cannabinoids appeared to decrease with highest values of applied nitrogen. Lowest overall concentrations of total THC and total CBD were seen at the highest (150 lbs N ac⁻¹) nitrogen rate whereas highest values were seen at the 75 lbs N ac⁻¹ rate. Differences in these two rates for total CBD showed a nearly 4% difference, while remaining with compliant ranges for total THC. From two years of evaluating nitrogen application rates, it does not appear that higher rates of nitrogen increase CBD or THC concentration and may in fact decrease overall potential cannabinoid concentration with higher rates. Under current regulations, major concerns are present with the available plant material for producing compliant crops under what could potentially be a wide array of growing conditions throughout the region. With such wide scale variations in growth habits, yield, and quality of various cultivars, it will be increasingly important to continue research and evaluation not only of available cultivars but also fertility practices to provide region specific information to optimize farmer yields within the Northeast. It is also important to note that only one variety and one fertility source was tested within this trial and other macronutrients or micronutrients could potentially impact cannabinoid profiles or expression under different growing conditions.

ACKNOWLEDGEMENTS

This project was supported by and was funded or partially funded through our partnership with CASE Institute and with Northeast SARE Partnership Grant award number ONE19-333. Special thanks to Roger Rainville and the staff at Borderview Research Farm for their generous help with the trials. We would also like to thank Catherine Davidson, Hillary Emick, Ivy Krezinski, Rory Malone, and Lindsey Ruhl for their assistance with data collection and entry. The information is presented with the understanding that no product discrimination is intended and no endorsement of any product mentioned or criticism of unnamed products is implied.

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