Screening hydrocarbon yields of sunflowers: *Helianthus maximiliani, H. grosseserratus H. nuttallii,* and *H. tuberosus* in the North Dakota-Minnesota-South Dakota area

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ABSTRACT

Analyses of biomass (g DW 10 leaves), % HC yields, and g HC yield/ g DW 10 leaves for four perennial sunflower species, *Helianthus grosseserratus, H. maximiliani, H. nuttallii ssp.* ssp. *rydbergii,* and *H. tuberosus* revealed that these taxa are all low in biomass in Minnesota, North Dakota, and South Dakota. Percent HC yields were lower (*H. grosseserratus* 5.42, *H. maximiliani* 3.18, *H. nuttallii ssp.* ssp. *rydbergii* 4.37, *H. tuberosus* 2.97%) than in annual, *H. annuus* in Texas. The small amount of biomass coupled with low % HC resulted in meager HC yields (g HC/ g DW leaves). However, a few high HC yielding plants were found: 7.76% *H. nuttallii*; 5.97% H. *grosseserratus*; 4.40% *H. tuberosus*; and 4.95% *H. maximiliani*.
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There has been considerable interest in bio-renewable sources of hydrocarbons (HC). Sunflowers have been surveyed on several occasions for hydrocarbons. Adams and Seiler (1984) surveyed 39 taxa of sunflowers for their cyclohexane (hydrocarbon) and methanol (resins) concentrations and reported the highest cyclohexane (bio-crude) yielding taxa were *H. agrestis*, an annual, Bradenton, FL (7.38%) and *H. annuus*, Winton, OK (7.09%). Further work by Adams et al. (1986) examined 614 plant taxa from the western US for their hydrocarbon (hexane soluble) and resin (methanol soluble) yields. They reported 2 plants of *H. annuus* from Idaho with 8.71% and 9.39% hydrocarbon yields. Recently, the survey of *H. annuus* was greatly expanded throughout the northcentral and southwestern US (Adams et al. 2017a; Adams et al. 2018a). Figure 1 (from Adams, et al. 2018a) shows the highest yielding plants were found in the Texas Panhandle (7.99%) with considerable geographical variation in % HC yields.

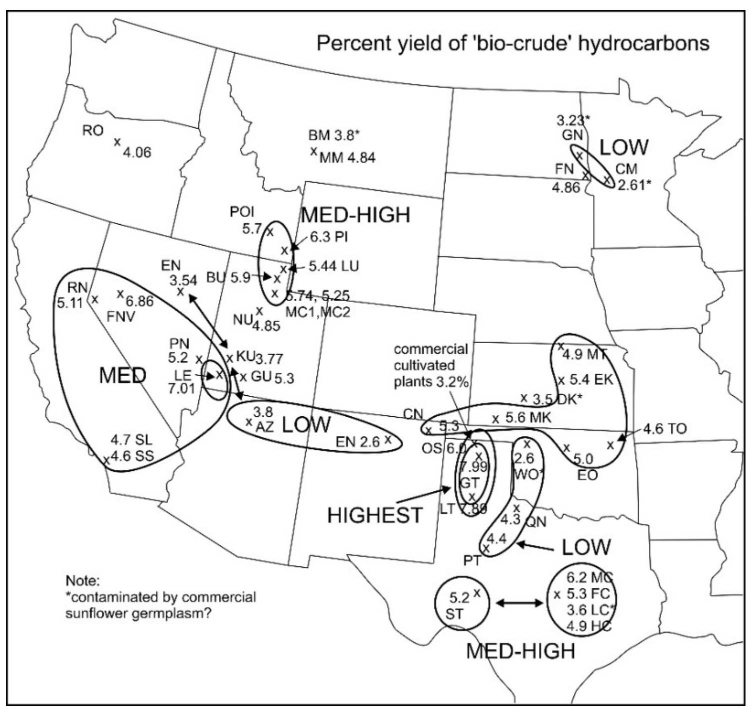

Figure 1. Geographic variation in the % HC in *H. annuus* (from Adams et al. 2018a).

Adams and Seiler (1984) surveyed 39 taxa of sunflowers that were grown in a common garden at the USDA lab, Bushland, TX. They analyzed cyclohexane (hydrocarbon), rubber, methanol (resins) yields plus protein concentrations. They reported cyclohexane (HC) yields for *H. maximiliani* of 3.10 - 3.50%; *H. nuttallii* 5.25% - 5.17%; *H. grosseserratus* 2.36 - 4.41% and *H. tuberosus* 2.26% (Table 1).

The purpose of the present study was to extensively examine variation in biomass, % HC yields and g HC/ biomass (g wt. 10 leaves) of four perennial sunflower species, *Helianthus grosseserratus, H. maximiliani, H. nuttallii ssp.* ssp. *rydbergii,* and *H. tuberosus* from a small geographic area in the North Dakota, Minnesota, South Dakota region. This report is a continuation of surveys on HC and rubber, and studies on the induction of HC in sunflowers (Adams and TeBeest, 2016; Adams et al. 2016, 2018c; Adams et al. 2017b).

Table 1. Analyses of 39 Taxa of *Helianthus*, representing 49 accessions. Modified from Adams and Seiler, 1984).

Helianthus species	Annual (A) or perennial (P)	Origin	C-hex fract.	Rubber yield[a]	MeOH fract.	Total	Percent Protein[b]
agrestis	A	Bradenton, FL	7.38	1.66	13.45	20.83	6.9
angustifolius	P	Alvin, TX	3.33	0.18	9.58	12.91	15.9
annuus	A	Winton, OK	7.09	1.40[c]	11.73	18.82	8.7
annuus, hybrid 894	A	Bushland, TX	2.23	0.49	14.65	16.88	8.6
anomalus	A	Mexican Water, AZ	5.74	0.18	12.30	18.04	9.8
agrophyllus	A	Rockport, TX	6.52	1.14[c]	9.60	16.12	11.9
arizonensis	P	Snowflake, AZ	6.13	0.28[c]	13.16	19.29	18.4
californicus	P	Napa, CA	3.05	1.78[c]	12.44	15.49	13.8
ciliaris	P	Bushland, TX	5.26	0.57	17.17	22.43	15.6
debilis	A	Titusville, FL	1.95	0.68	8.83	10.78	9.6
deserticola	A	Leeds, UT	3.16	0.82	10.96	14.12	5.3
divaricatus	P	Wister, OK	1.09	0.47[d]	11.54	13.44	2.6
glaucophyllus	P	Blowing Rock, NC	3.29	0.25	9.50	12.79	8.1
grosseserratus	**P**	**Cherokee Co., KS**	**2.36**	**0.28**	**12.28**	**14.64**	**14.6**
grosseserratus	**P**	**Hooker Co., KS**	**4.41**	**0.28**	**14.37**	**18.78**	**20.1**
grosseserratus	**P**	**Stuart, OK**	**3.56**	**0.28**	**10.49**	**14.05**	**17.1**
hirsutus	P	Wilburton. OK	1.60	0.30	8.30	9.90	6.1
laciniatus	P	Mimbres River, NM	3.15	0.31	12.40	15.55	9.9
laetiflorus	P	Lyon Co., KS	2.22	0.66	10.64	12.86	11.9
laevigatus	P	Botetourt Co., VA	3.53	na	18.24	21.77	13.9
maximiliani	**P**	**Bloomington, IN**	**3.10**	**na**	**13.21**	**16.31**	**10.8**
maximiliani	**P**	**San Jon, NM**	**3.50**	**0.24**	**9.87**	**13.37**	**15.3**
maximiliani	P	Gatesville, TX	2.53	na	10.30	12.83	8.9
microcephalus	P	Cherokee Co., SC	4.77	0.26[c]	14.25	19.02	14.1
mollis	P	Greenwood Co., KS	3.26	0.31	11.05	14.31	8.9
mollis	P	Okmulgee Co., OK	2.60	0.31	9.72	12.32	8.5
mollis	P	Rivercrest, TX	1.87	0.31	8.58	10.45	6.6
neglectus	A	Kermit, TX	3.83	0.10	11.71	15.54	16.2
nuttallii	**P**	**Orovada, NV**	**5.25**	**0·96[c]**	**10.23**	**15.48**	**8.8**
nuttallii	**P**	**Payson, UT**	**5.17**	**na**	**12.76**	**17.93**	**10.6**
occidentalis	P	Raymondville, MO	2.12	0.48	15.14	17.26	11.9
occid. ssp. *plantagineus*	P	Sheridan, TX	2.36	1.62	18.33	20.69	8.8
paradoxus	A	Ft. Stockton, TX	3.46	0.15	19.54	23.00	13.3
petiolaris ssp. *fallax*	A	Adrian, TX	2.15	0.30	11.99	14.14	17.3
petiolaris ssp. *petiolaris*	A	Memphis, TX	1.86	0.14	21.00	22.86	12.1
praecox ssp. *hirtus*	A	Carrizo Springs, TX	5.19	0.49	10.05	15.24	13.8
pumilus	P	Boulder, CO	1.72	0.53	6.87	8.59	7.4

resinosus	P	Collins, MS	2.89	1.78[c]	11.76	14.65	11.9
rigidus ssp. *rigidus*	P	Brookston, IN	1·86	na	9.93	11.79	7.8
rigidus ssp. *subrhomboides*	P	Leyden, CO	1·42	na	10.90	12.32	9.9
salicifolius	P	Kansas	3.13	0.37	9.30	12.43	7.1
salicifolius	P	Muenster, TX	3.26	0.37	9.31	12.57	11.2
silphioides	P	Wister, OK	2.63	0.42	18.01	20.64	10.0
simulans	P	Milton, FL	3.42	0.31	13.91	17.33	18.1
smithii	P	Morgantown, NC	4.48	0.58[c]	11.77	16.25	12.2
strumosus	P	Siler City, |NC	2.98	0.55	11.80	14.78	12.9
tuberosus	**P**	**Kilgore, TX**	**2.26**	**0.93**	**13.28**	**15.54**	**12.1**
tuberosus x *annuus*	P	Turlock, CA	1.73	na	12.21	13.94	9.3
Average			3.39	0.57	12.26	15.65	11.35

a Rubber yields, for leaves except for *divaricatus,* are from Stipanovic et al. 1980. *b* Protein determined by Kjeldahl N x 6-25. *c* By 13C·NMR spectral analysis. All others by gravimetric. *d* Whole plant analyzed.

MATERIALS AND METHODS

Population locations - see Appendix I. Ten or sometimes 20 mature leaves were collected at stage R 5.1- 5.3, that is, when first flower head has opened with mature rays. Leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). 3 g of air-dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared. Raw yields were corrected by a correction factor (CF) that was developed from data obtained by performing a soxhlet, 6 hr extraction. The correction factor = soxhlet hexane yields divided by yields from 18 hr shaking in hexane = 2.06. Analysis of variance (ANOVA) and SNK (Student-Newman-Keuls) multiple range tests were performed based on the formulations in Steel and Torrie (1960).

RESULTS

Analyses of biomass (g dw 10 leaves), % HC yields and g HC yield/ 10 leaves are shown for 17 populations of *H. maximiliani* in Table 2. Biomass is small due to the small leaves and ranged from 3.37 g / 10 lvs. (Moorhead, MN) to only 1.54 g (Milnor, ND). The % HC yields were not very large and varied from 3.85% (x 12. Harwood, ND) to 2.23% (x 15, Oaks, ND, Fig. 2). There was not much of a regional trend in HC yields (Fig. 2), so perhaps the variation was mostly due to edaphic factors, rather than genetic factors.

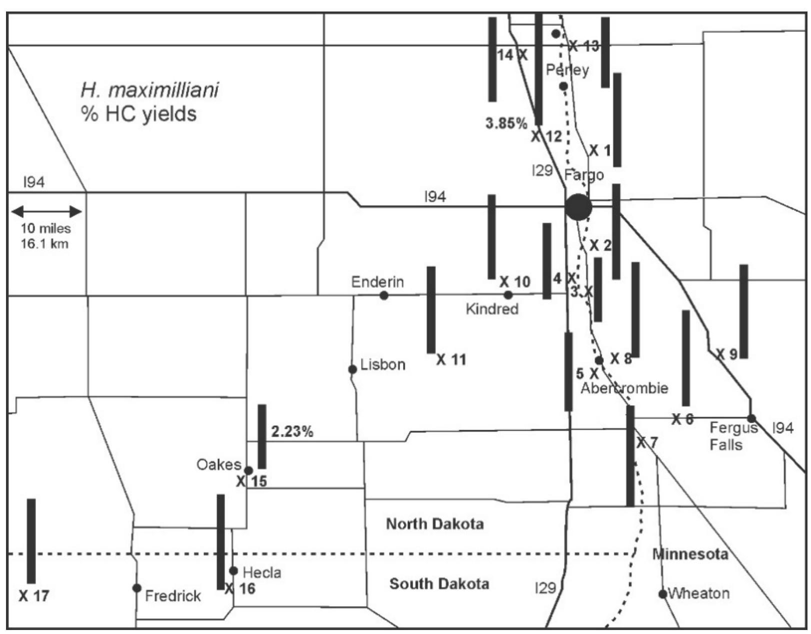

Figure 2. Distribution of % HC yields for *H. maximiliani.*

Table 2. The yields biomass (g dw, 10 lvs), % HC yield, g HC/ g 10 lvs for *H. maximiliani*. Any two means in a column with the same suffix letter are **not** significantly different (P= 0.05).

H. maximiliani popn. IDs	g wt / 10 lvs	% HC yield	Coef. of var. %	Range of yields	g HC/ plant (10 lvs)	Site information
ANOVA F ratio, signif. Probability, P	15.6*** $3.7x10^{-7}$	7.1*** $6.2x10^{-5}$			16.1*** $3.0x10^{-7}$	
12 Harwood, ND	2.28cd	**3.85m**	7.06	3.40-4.22	0.088y	North of Harwood, ND, dry roadside ditch near RR tracks, ~40 plants near some trees.
M7 Milnor, ND	1.54f	3.59mn	4.90	3.30-3.81	0.056yz	West of Milnor, ND, dry roadside ditch next to soybean field, ~50 scattered plants.
M2 Comstock, MN	1.85cdef	3.41mno	19.40	2.33-4.94	0.062yz	North of Comstock, MN, dry undisturbed roadside ditch, next to soybean field, 20-30 plants.
M6 DeLamere, ND	1.79cdef	3.40mno	5.44	3.09-3.61	0.061yz	South of DeLamere, ND, dry roadside ditch next to soybean field, 30 plants.
M8 Lisbon, ND	1.88cdef	3.38mno	8.02	3.09-3.81	0.064y	East of Lisbon, ND, dry roadside ditch next to soybean field, 40-50 plants.
16 Hecla, SD	2.23bcde	3.38mno	7.63	2.98-3.71	0.076y	South of Hecla, SD, dry roadside ditch but seasonally moist, 20 plants.
M9 McLeod, ND	2.32cd	3.34mno	5.33	2.99-3.50	0.078y	Northwest of McLeod, ND, dry roadside ditch, scattered population of ~100 plants.
M1 Moorhead, MN	**3.37a**	3.32mno	12.98	2.74-4.12	0.112x	North of Moorhead, MN, roadside ditch along RR tracks, dry undisturbed area, 100 scattered plants.
11 Sheldon, ND	1.82cdef	3.11mnop	13.67	2.37-3.40	0.056yz	South of Sheldon, ND, dry roadside ditch next to corn field, 30-40 scattered plants.
10 Kindred, ND	2.2cd8	2.99nop	6.59	2.78-3.30	0.068y	West of Kindred, ND, Upper dry slopes of roadside drainage ditch, 50 plants.
14 Kelso, ND	2.04cdef	2.99nop	4.33	2.78-3.19	0.061yz	South of Kelso, ND, seasonally moist roadside ditch, ~100 plants scatter in ditch.
17 New Effington, SD	2.21bcde	2.98nop	6.12	2.78-3.30	0.066y	East of New Effington, SD, roadside ditch near powerline R/W, seasonally moist cut over area, ~200 plants
M5 Abercrombie ND	1.99cdef	2.81nopqr	12.70	2.40-3.43	0.113x	North of Abercrombie, ND, dry roadside ditch next to soybean field, 75 scattered plants.
M4 Lithia, ND	2.69b	2.72opqr	7.65	2.40-3.02	**0.145w**	North of Lithia, ND, dry roadside ditch next to sunflower field, 25 plants
13 Hendrum, MN	2.17bcde	2.51pqr	6.14	2.37-2.78	0.055yz	North of Hendrum, MN, dry roadside ditch near old RR track, ~ 100 scattered plants.
M3 Wolverton, MN	2.74b	2.28qr	26.27	1.51-3.18	0.064y	North of Wolverton, MN, dry undisturbed roadside ditch along fence row next to soybean field, 50-60 plants.
15 Oaks, ND	1.64ef	2.23r	6.28	2.06-2.37	0.037z	West of Oaks, ND, dry roadside ditch, ~150 plants.
Results from other *H. maximiliani* populations (from Adams et al. 2018b)						
MXOK	2.43	**5.06**	26.8	3.16-7.82	0.123	15333, *H. maximiliani,* Langston, OK, K. Hart
MXMT	1.43	3.71	9.5	3.32-4.47	0.053	15276, *H. maximiliani,* Bozeman, MT M. Lavin, wet cattail area, dry in summer
MxMC	**4.98**	3.68	24.7	2.54-5.42	**0.180**	15342, *H. maximiliani,* McLennan Co., TX W. Holmes
MxCC	4.25	3.24	30.0	2.17-5.42	0.138	15340, *H. maximiliani,* Coryell Co., TX W. Holmes,
MxFC	4.04	2.20	24.3	1.19-3.17	0.089	15341, *H. maximiliani,* Falls Co., TX W. Holmes,

Some of the populations were quite variable with a COV (Coefficient Of Variation) that ranged from 26.27% (Wolverton, MN, x 3) to a low of 4.33% near Kelso, ND (x 14, Table 2). The largest % yield was a plant with 4.94% in the Comstock, MN (x 2) population. Yields of HC, as g/ g DW 10 leaves, varied from 0.145 (x 4), Lithia, ND down to 0.037 (x 15), Oaks, ND (Table 2).

High HC yielding plants of *H. maximiliani* (from Adams et al. 2018b) are shown at the bottom of Table 2 for comparisons. The Langston, OK population (Table 2) has a % HC yield of 5.06% that is much higher than found in this study. However, the other *H. maximiliani* populations sampled have somewhat lower HC yields. The McLennan Co. TX population had high biomass (4.98g) and a high HC g wt yield (0.180, Table 2).

Analyses of % HC yields of *H. nuttallii* ssp. *rydbergii* populations (Fig. 3) revealed that highest yield was from the Kindred, ND area (x 3, 6.54%) and the lowest yield, 2.61% was nearby (x 2). The population near Kindred (x 3) appears to be the most unusual population sampled in having larger % HC yields. This may be due to some type of microhabitat at that site.

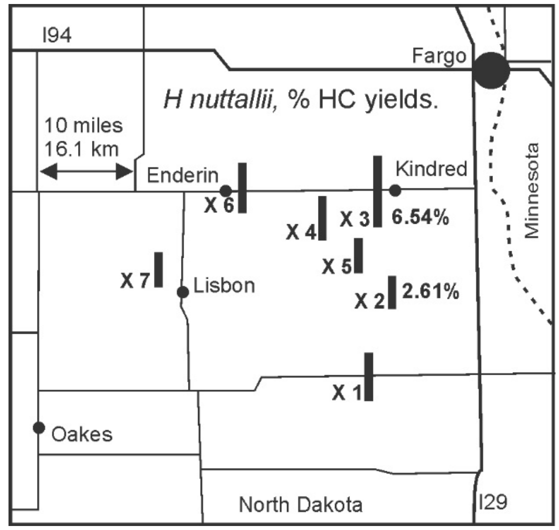

Figure 3. % HC yields for *H. nuttallii*.

Table 3. The yields biomass (g dw, 10 lvs), % HC yield, and g HC/ g 10 lvs for *H. nuttallii* ssp. *rydbergii*. Any two means in a column with the same suffix letter are **not** significantly different (P= 0.05).

H. nuttallii ssp. *rydbergii* popn. IDs	g wt / 10 lvs	% HC yield	Coef. of var. %	Range of yields	g HC/ plant (10 lvs)	Site information
ANOVA F ratio, signif. Probability, P	78.1*** $1.4x10^{-6}$	53.5*** $3.8x10^{-7}$			16.0*** $3.4x10^{-4}$	
N3 Kindred, ND	2.05d	**6.54**g	11.08	5.71-**7.76**	**0.134**x	Southwest of Kindred, ND, moist swampy roadside ditch near the Sheyenne National Grasslands, large scattered population of ~200 plants.
N6 Enderlin, ND	2.42bc	5.21h	6.92	4.64-5.77	0.127x	East of Enderlin, ND, swampy roadside ditch, very large population of ~300 scattered plants.
N1 DeLamere ND	1.32f	5.01h	6.14	4.78-5.62	0.067z	South of DeLamere, ND, moist swampy roadside ditch next to soybean field, 30 plants.
N4 Leonard, ND,	2.67c	4.53i	10.86	3.91-5.15	0.103xy	South of Leonard, ND, swampy roadside ditch near sunflower field, very large scattered population of ~500 plants.
N5 Lisbon, ND	2.72b	3.56j	8.29	3.09-3.91	0.098y	West of Lisbon, ND, swampy roadside ditch, scattered population of 30-40 plants.
N7 Marion, ND	1.34e	3.55j	5.20	3.30-3.82	0.047z	Southwest of Marion, ND, swampy roadside ditch, edge of cattail slough, large population extending ½ mile.
N2 Wyndmere ND	**4.14**a	2.61k	8.60	2.27-2.88	0.108xy	North of Wyndmere, ND, roadside ditch moist swampy area, near the Cheyenne National Grasslands, scattered population of ~75-100 plants.
Results from other *H. maximiliani* populations (from Adams et al. 2018b)						
NuGUT	**2.23**	**7.02**	16.4	5.58-**9.16**	**0.156**	15260, *H. nuttallii,* Glendale UT Adams, wet ditch, dry in summer

NuSAZ	2.03	5.43	9.5	4.49-6.07	0.110	15290, *H. nuttallii,* Licher, Sedona, AZ,
NuKUT	1.75	3.12	37.7	1.7-5.26	0.055	15263, *H. nuttallii,* Kanab, UT Adams wet bank of spring fed pond.

Analyses of % HC yields of *H. tuberosus* populations (Fig. 4) revealed that highest yield was from north of Perley, MN (x 6, 3.79%) and the lowest yield was from x 2 (1.98%, Fig. 4). The higher yielding populations are grouped around Perley. The highest yielding individual, 4.40%, was from the Perley (x 6) population.

It might be noted that the single population of *H. grosseserratus*, in South Dakota (Gros, Fig. 4), was quite high in % HC (5.42%). The coefficient of variation in % HC yields was not large (several populations from 11.50 - 16.51% (Table 4).

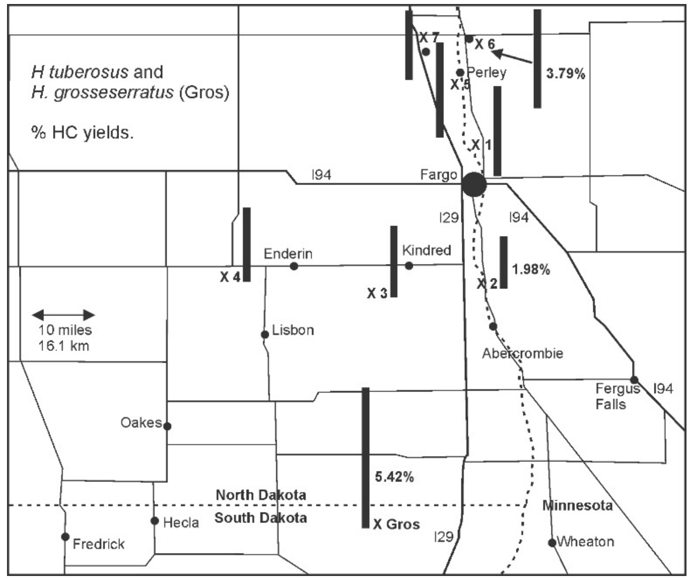

Figure 4. Distribution of % HC yields of H. *tuberosus* and a single population of *H. grosseserratus* (Gros).

Leaf biomass was considerably larger for *H. tuberosus* due to larger leaves and ranged from 9.50 down to 4.98 g/ g DW 10 leaves (Table 4). This is approximately 3 times larger than found in *H. maximiliani*, or *H. nuttallii*. This resulted in very large yields (north of Perley, 0.294 and east of Perley 0.261 g/ g DW 10 leaves, Table 4). *Helianthus tuberosus* (Jerusalem artichoke) can be cultivated for inulin in the tubers that can be converted to fructose and thence to ethanol (Sachs et al. 1981). Jerusalem artichoke can also produce a significant amount of above ground biomass that can be converted to fuels by cellulosic digestion (Duvniak et al. 1991). Seiler (1993) reported forage yields of Jerusalem artichoke (JA) cultivars grown in Texas sampled at flowering varied from 3.0 to 6.3 mg ha^{-1}, with the cultivated JA 'Sunchoke' having the highest yield at 6.3 Mg/ha, while at maturity, cultivar 'Vadim' had the highest mean tuber yield of 9.04 mg/ha, making it a potential multi-purpose biofuel crop.

We also compared the biomass and HC yields for the taxa surveyed in this study with some populations of *H. annuus* from previous studies (Table 5). Notice that among the four taxa in this study, biomass was significantly larger in *H. tuberosus*, but % HC yield and g HC/ plant were the highest in *H. grosseserratus* based on a single population.

Examination of the biomass and yields from *H. annuus* from 3 high yielding populations (Table 5) shows that their biomass was 2.5 to 5 times as large as that of *H. maximiliani, H. nuttallii, H. tuberosus* and *H. grosseserratus*. The % HC yields from *H. annuus* from natural populations were large (5.75 - 7.99 %) and correspondingly, the g HC/ g 10 vs. was much higher than the four perennial taxa in this study because they have mostly relatively small leaves.

Table 4. The yields biomass (g dw, 10 lvs), % HC yield, and g HC/ g 10 lvs for *H. tuberosus* and *H. grosseserratus*. Any two means in a column with the same suffix letter are **not** significantly different (P= 0.05).

H. tuberosus popn. IDs	g wt / 10 lvs	% HC yield	Coef. of var. %	Range of yields	g HC/ plant (10 lvs)	Site information
ANOVA F ratio, signif. Probability, P	 42.3*** $7.8x10^{-6}$	 15.9*** $2.6x10^{-4}$			 19.0*** $1.6x10^{-4}$	
T6 north Perley, MN	7.13c	**3.79**g	12.13	3.09-4.40	**0.261**x	North of Perley, MN, swampy area, flood plain of Wild Rice River, small popn. 30 plants.
T5 east Perley, MN	8.20b	**3.63**g	3.93	3.43-3.78	**0.294**x	East of Perley, MN, moist river bank of the Red River of the North near bridge, ~45 plants.
T1 Moorhead, MN,	5.60de	**3.46**g	16.51	2.78-4.33	0.197y	North of Moorhead, MN, roadside ditch near bridge of drainage ditch, moist area along edge of trees, 40-50 plants.
T4 Enderlin, ND	6.10d	2.84h	8.17	2.47-3.09	0.174y	East of Enderlin, ND, swampy roadside ditch, small scattered population of 30 plants.
T3 Kindred, ND	5.55de	2.74h	13.43	2.34-3.30	0.150yz	Southwest of Kindred, ND, swampy roadside ditch near sunflower field, large population ~200 plants scattered for several hundred feet.
T7 Kelso, ND	4.98e	2.36hi	4.65	2.20-2.54	0.118z	North of Kelso, ND, moist roadside with 50 plants scattered along the edge of trees.
T2 Christine, ND	**9.50**a	1.98i	11.50	1.72-2.34	0.186y	East of Christine, ND, roadside ditch near bridge of the Red River of the north, moist bank area along edge of trees, ~200 plants.
***H. grosseserratus*, Veblen, SD**						
GRO, Veblen, SD	5.35	**5.42**	5.19	4.94-5.97	**0.292**	East of Veblen, SD, seasonally moist roadside drainage area with cattails, scattered population on both sides of road, ~100 plants

Table 5. The yields of biomass (g dw, 10 lvs), % HC yield, and g HC/ g 10 lvs for *H. maximiliani, H. nuttallii, H. tuberosus* and *H. grosseserratus* compared with *H. annuus* (from Adams et al. 2017b). Any two means in a column with the same suffix letter are **not** significantly different (P= 0.05).

Species	biomass g wt / g wt 10 lvs	% HC yield	g HC/ plant (10 lvs)
ANOVA: F ratio, significance Probability, P =	147.7*** $9.5x10^{-10}$	39.4*** $7.2x10^{-7}$	107.7*** $3.8x10^{-9}$
H. maximiliani	2.20c	3.18i	0.076z
H. nuttallii	2.31c	4.37h	0.095z
H. tuberosus	**6.72**a	2.97i	0.197y
H. grosseserratus	5.35b	**5.42**g	**0.292**x
***H. annuus*, natural vs. seeds from that population grown in greenhouse**			
H. annuus, ex natural Gruver, TX	**19.36**	**7.99**	**1.499**
H. annuus, greenhouse plants grown from seed from Gruver, TX	2.54 **(13.1%)**	3.67 **(45.9%)**	0.092 **(6.1%)**
H. annuus, natural Lake Tanglewood, TX	**19.11**	**7.88**	**1.484**
H. annuus, , greenhouse plants grown from seed from Lake Tanglewood, TX	2.79 **(14.6%)**	4.38 **(55.6%)**	0.120 **(8.1%)**
H. annuus, natural Salt Lake City, UT	**11.92**	5.75	0.672
H. annuus, greenhouse plants grown from seed from Salt Lake City, UT,	2.69 **(22.6%)**	4.50 **(78.3%)**	0.121 **(17.9%)**

However, one of the perplexing factors in *H. annuus* is the strong environmental component present when plants are grown in a greenhouse. Notice that % HC yields decreased in the greenhouse grown plants. In fact, % HC yields were only 45.9, 55.6, and 78.3% in the greenhouse grown vs. naturally grown plants (Table 5). The Texas populations (Gruver, TX, Lake Tanglewood, TX) in the Texas Panhandle were very stressed with wilted leaves and leaves eaten by grasshoppers and other insects. It may be that higher HC production was induced in these populations. At present, research involving stressing greenhouse plants by the application of growth regulators have not revealed any chemical that can induce high HC yields (Adams and Johnson, 2018; 2019; Adams et al. 2016; 2017b).

Finally, it should be noted that *H. maximiliani, H. nuttallii, H. tuberosus* and *H. grosseserratus* are grow in seasonally wet to swampy habitats (see Appendix I, Site information). But, *H tuberosus* can be cultivated in drier soils, so it may have some potential as a multi-product crop.

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Appendix I. Locations of populations of *H. maximiliani, H. grosseserratus, H. nuttallii and H. tuberosus* sampled in this study.

H. maximiliani popn. IDs	samples/ popn.	Site information	County	Latitude	Longitude
M1 MAX MM, Moorhead, MN	9	North of Moorhead, MN, roadside ditch along RR tracks, dry undisturbed area, 100 scattered plants.	Clay, MN	N 46.97702	W 96.74728
M2 B3 MAX CM, Comstock, MN	9	North of Comstock, MN, dry undisturbed roadside ditch, next to soybean field, 20-30 plants.	Clay, MN	N 46.73838	W 96.76250
M3 B4 MAX WM, Wolverton, MN	5	North of Wolverton, MN, dry undisturbed roadside ditch along fence row next to soybean field, 50-60 plants.	Wilkin, MN	N 46.59403	W 96.74290
M4 B6 MAX LtN, Lithia, ND	5	North of Lithia, ND, dry roadside ditch next to sunflower field, 25 plants	Richland, ND	N 46.63033	W 96.82085
M5 B7 MAX AN Abercrombie, ND	5	North of Abercrombie, ND, dry roadside ditch next to soybean field, 75 scattered plants.	Richland, ND	N 46.45640	W 96.73614
M6 B9 MAX DN, DeLamere, ND	5	South of DeLamere, ND, dry roadside ditch next to soybean field, 30 plants.	Sargent, ND	N 46.23442	W 97.32183
M7 B10 MAX MLN, Milnor, ND	5	West of Milnor, ND, dry roadside ditch next to soybean field, ~50 scattered plants.	Sargent, ND	N 46.22578	W 97.54917
M8 B11 MAX LbN, Lisbon, ND	5	East of Lisbon, ND, dry roadside ditch next to soybean field, 40-50 plants.	Ransom, ND	N 46.44221	W 97.63865
M9 B12 MAX McN, McLeod, ND	5	Northwest of McLeod, ND, dry roadside ditch, scattered population of ~100 plants.	Ransom, ND	N 46.44226	W 97.32230
10 B14 MAX KN, Kindred, ND	5	West of Kindred, ND, Upper dry slopes of roadside drainage ditch, 50 plants.	Cass, ND	N 46.62954	W 97.07226
11 B19 MAX SN, Sheldon, ND	5	South of Sheldon, ND, dry roadside ditch next to corn field, 30-40 scattered plants.	Ransom, ND	N 46.47187	W 97.48933
12 B22 MAX HN, Harwood, ND	5	North of Harwood, ND, dry roadside ditch near RR tracks, ~40 plants near some trees.	Cass, ND	N 46.97702	W 96.89918
13B25 MAX HM, Hendrum, MN	5	North of Hendrum, MN, dry roadside ditch near old RR track,~ 100 scattered plants.	Norman, MN	N 47.30009	W 96.81122
14B27 MAX KN, Kelso, ND	5	South of Kelso, ND, seasonally moist roadside ditch, ~100 plants scatter in ditch.	Traill, ND	N 47.30401	W 97.02623
15 B29 MAX ON, Oaks, ND	5	West of Oaks, ND, dry roadside ditch, ~150 plants.	Dickey, ND	N 46.13889	W 98.14533
16 B30 MAX HS, Hecla, SD	5	South of Hecla, SD, dry roadside ditch but seasonally moist, 20 plants.	Brown, SD	N 45.79483	W 98.14483
17 B32 MAX NES, New Effington, SD	5	East of New Effington, SD, roadside ditch near powerline R/W, seasonally moist cut over area, ~200 plants	Roberts, SD	N 45.84889	W 96.89655

H. nuttallii popn. IDs	samples/ popn.	Site information	County	Latitude	Longitude
N1 B8 NUT (*nuttallii* ssp. *rydbergii*)	5	South of DeLamere, ND, moist swampy roadside ditch next to soybean field, 30 plants.	Sargent, ND	N 46.22195	W 97.32242
N2 B13 NUT (*nuttallii* ssp. *rydbergii*)	5	North of Wyndmere, ND, roadside ditch moist swampy area, near the Sheyenne National Grasslands, scattered population of ~75-100 plants.	Richland, ND	N 46.39833	W 97.13411

N3 B15 NUT (*nuttallii* ssp. *rydbergii*)	5	Southwest of Kindred, ND, moist swampy roadside ditch near the Sheyenne National Grasslands, large scattered population of ~200 plants.	Richland, ND	N 46.58162	W 97.13760
N4 B17 NUT (*nuttallii* ssp. *rydbergii*)	5	South of Leonard, ND, swampy roadside ditch near sunflower field, very large scattered population of ~500 plants.	Richland, ND	N 46.56163	W 97.21896
N5 B18 NUT (*nuttallii* ssp. *rydbergii*)	5	West of Lisbon, ND, swampy roadside ditch, scattered population of 30-40 plants.	Richland, ND	N 46.45131	W 97.21825
N6 B20 NUT (*nuttallii* ssp. *rydbergii*)	5	West of Enderlin, ND, swampy roadside ditch, very large population of ~300 scattered plants.	Ransom, ND	N 46.62957	W 97.52242
N7 B28 NUT (*nuttallii* ssp. *rydbergii*)	5	Southwest of Marion, ND, swampy roadside ditch, edge of cattail slough, large population extending ½ mile.	LaMoure, ND	N 46.48585	W 98.15190

H. grosseserratus popn. IDs					
G1 B31 (*grosseserratus*)	10	East of Veblen, SD, seasonally moist roadside drainage area with cattails, scattered population on both sides of road, ~100 plants.	Marshall, SD	N 45.86354	W 97.32122

H. tuberosus popn. IDs	samples /popn.	Site information	County	Latitude	Longitude
T1 B1 TUB MM, Moorhead, MN	5	North of Moorhead, MN, roadside ditch near bridge of drainage ditch, moist area along edge of trees, 40-50 plants.	Clay, MN	N 46.94864	W 96.77061
T2 B5 CN, Christine, ND	5	East of Christine, ND, roadside ditch near bridge of the Red River of the north, moist bank area along edge of trees, ~200 plants.	Richland, ND	N 46.57238	W 96.75393
T3 B16 TUB KdN, Kindred, ND	5	Southwest of Kindred, ND, swampy roadside ditch near sunflower field, large population of ~200 plants scattered for several hundred feet.	Richland, ND	N 46.57271	W 97.14014
T4 B21 TUB EN, Enderlin, ND	5	East of Enderlin, ND, swampy roadside ditch, small scattered population of 30 plants	Barnes, ND	N 46.63010	W 97.87658
T5 B23 TUB ePM, Perley, MN	5	East of Perley, MN, moist river bank of the Red River of the North near bridge, ~45 plants.	Norman, MN	N 47.17957	W 96.82209
T6 B24 TUB nPM, Perley, MN)	5	North of Perley, MN, swampy area, flood plain of Wild Rice River, small population of 30 plants.	Norman, MN	N 47.28012	W 96.81380
T7 B26 TUB KsN, Kelso, ND	5	North of Kelso, ND, moist roadside with 50 plants scattered along the edge of trees.	Traill, ND	N 47.32582	W 97.03096