The effects extreme alkaline soil on biomass and hydrocarbon yields in *Helianthus annuus* cv. Munchkin, Firecracker and Little Becka (Asteraceae, Sunflowers)

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ABSTRACT

Sunflowers, *H. annuus* cv. Munchkin, Firecracker and Little Becka, were greenhouse grown in alkaline soil, common in St. George, UT, to determine its effects on leaf biomass, percent yields of free hydrocarbons (HC), and yields of gHC/ g biomass. Biomass was very reduced, but was 40 - 60% larger for Firecracker and Little Becka than for Munchkin. Percent (%) hydrocarbon (HC) yield was largest in Little Becka (4.14%). with Firecracker and Munchkin having significantly lower yields (3.47 - 3.16%). Yields as g HC/ g 10 leaves were very small with only 0.05 - 0.06 g for Little Becka and Firecracker and only 0.03g for Munchkin. Comparison with a previous study of Munchkin revealed the biomass, % HC yield, and gHC/ g 10 leaves in Munchkin grown in alkaline soil was far lower than even plants grown in potting soil watered with 120 mM salt or 240 mM salt solutions. In contrast to salt (NaCl) stress, alkaline stress did not result in an increase of HC yields. Published on-line www.phytologia.org *Phytologia 102(3): 143-149 (Sept 21, 2020).* ISSN 030319430.
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A recent study examined the effects of watering using increasing concentrations of salt and pure sand on HC (hydrocarbon) yields and biomass (Johnson, Theobald and Adams 2019). They found biomass, % HC yields, and g HC yields all declined, from the control, to 30 mM, and 120 mM salt (red ellipses, Fig. 1); however, at 240 mM salt, the % HC yield increased (Fig. 1). An unusual trend was seen in the sand treatment (nutrient deficiency) where Munchkin % HC yield increased, but its biomass was very low (Fig. 1). The total g HC yields were not significantly different among the wide range of salt concentrations utilized in that study (yellow ellipse, Fig. 1). Considering that seawater is approximately 600 mM salinity, the 240 mM salt water is a very high salt concentration (~ 40% of seawater). Although less biomass would be produced, total g HC yields (yellow ellipse, Fig. 1) were not significantly different, even with increasingly higher salt concentrations.

Defense chemicals are both constitutive and inducible defenses (see Whittstock and Gershenzon, 2002 for discussion). Recently, we reported (Adams et al. 2017c) that progeny of high hydrocarbon (HC) yielding sunflower (*H. annuus*) populations displayed much reduced HC yields when grown in greenhouse conditions. We reported the percent HC (greenhouse / field grown HC yields) decreased to 45.9, 55.6 and 78.3%. In addition, g HC / g DW weights of leaves were very reduced to from 17.9 g to 6.1 g when plants were grown in a greenhouse. It appears that biotic and abiotic factors in natural populations can have large effects on HC yields.

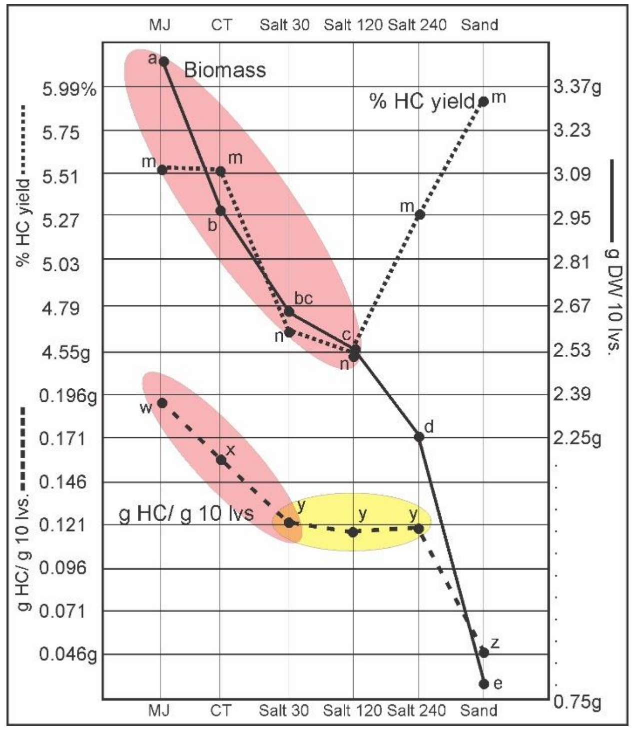

Figure 1. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ g DW 10 leaves for Munchkin subjected to 5 treatments. Means with the same letter superscripts are **not** significantly different (P= 0.05). Similar trends are noted by the red ellipses. The yellow ellipse highlights the uniform HC yields from plants watered with increasing salt concentrations (30 mM, 120 mM, 240 mM) of saltwater. See text for discussion.

The purpose of the present paper is to report the effects nutrient-poor alkaline soil, typical of St. George, Utah, on biomass, % HC yields and gHC/ g DW leaves for 3 sunflower cultivars: Munchkin, Little Becka and Firecracker (*Helianthus annuus* cultivars). This report is a part of a continuing study on the development of sunflowers as a source for natural rubber and bio-fuels from the biomass (Adams et al., 1986; Adams and Seiler, 1984; Adams and TeBeest, 2016; Adams et al. 2016; Adams and TeBeest, 2017; Adams et al. 2017a,b,c; Adams and Johnson 2018; Adams et al. 2018a,b,c; Johnson et al. 2019; Pearson et al., 2010a,b; Seiler, Carr and Bagby, 1991,).

MATERIALS AND METHODS

Seeds of *H. annuus* cv. Munchkin , Firecracker and Little Becka were obtained from Sunflower Selections, Inc., Woodland, CA. Seeds were germinated in potting soil in 2" square cups in a lab growth chamber, then one week after germination they were transplanted into 6 " square plastic pots using two kinds of alkaline soils from St. George, UT. Soil testing was by A & L labs, Modesto, CA. Two sets of 42 plants (14 each of Munchkin, Firecracker and Little Becka) were grown in the greenhouse at Pine View High School (PVHS), St. George, UT from Feb. 15, 2020 until March 15, 2020 when the CovID-19 virus led to closing the school. The plants were then transferred to a growth chamber with LED lighting approximately equal to daylight for 16 hr light, 8 hr dark cycles. Plants were watered with 200 ml tap water twice per week in the PVHS greenhouse. After moving to the lab growth chamber, plants were watered when wilted leaves appeared. The 10 largest, non-yellowed, mature leaves were collected. The 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 (or less in some cases) of air-dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar was sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was filtered through a Whatman (P8) filter paper into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pre-weighed aluminum pan with concentrated hydrocarbon extract was weighed and tared. Extraction of identical samples by shaking and soxhlet (8 hr) yielded a correction factor of 1.9 (soxhlet yield/ shaking yield), which when corrected to oven dry weight basis (ODW) by 1.085 resulted in a total correction factor of 2.06. ANOVA and SNK (Student Newman-Keuls) multiple range tests were programmed following the formulations in Steel and Torrie (1960).

RESULTS

Table 1 shows the results from the treatments ANOVA and SNK statistical analyses. Biomass was highly significantly larger in Little Becka and Firecracker, than in Munchkin (Table 1, Fig. 2). Previously, we found (Johnson et al. 2019) biomass from Munchkin grown in potting soil is about 3g (Fig. 1) vs. 0.965 g in this study (Table 1). Percent (%) HC yields were significantly higher in Little Becka (4.14%) (Table 1). Yield of HC (as gHC/ g DW 10 leaves) was significantly higher in Firecracker and Little Becka, but, of course, very small due to the small amount of biomass (Table 1,Fig. 2).

Table 1. Comparison of dry weight (10 leaves), in % HC yields, and g HC/ g DW 10 leaves for cv. Munchkin, Firecracker and Little Becka grown on alkaline soil. Mean values with the **same suffix letter** (gray highlighted) are **not** significantly different (P= 0.05).

	Munchkin	Little Becka	Firecracker	F ratio, significance
Biomass, g DW 10 leaves	0.965a	1.41b	1.55b	F= 6.104, P = 0.007 ***
	Munchkin	Firecracker	Little Becka	F ratio, significance
% HC yield	3.16 e	3.47e	4.14f	F= 9.098, P = 0.00125***
	Munchkin	Firecracker	Little Becka	F ratio, significance
g HC/ g DW 10 leaves	0.030y	0.053z	0.058z	F= 9.683, P = 0.946***

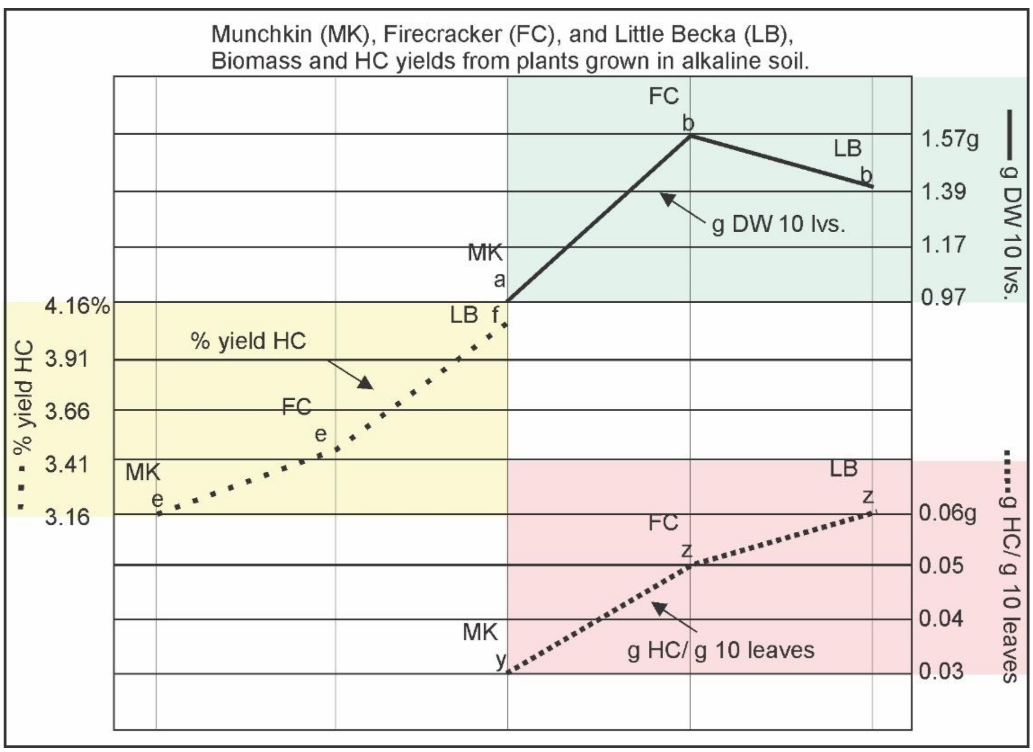

Figure 2. Graphs of biomass, % HC yield, and g HC/ g DW 10 leaves for Munchkin, Firecracker and Little Becka plants grown in alkaline soil.

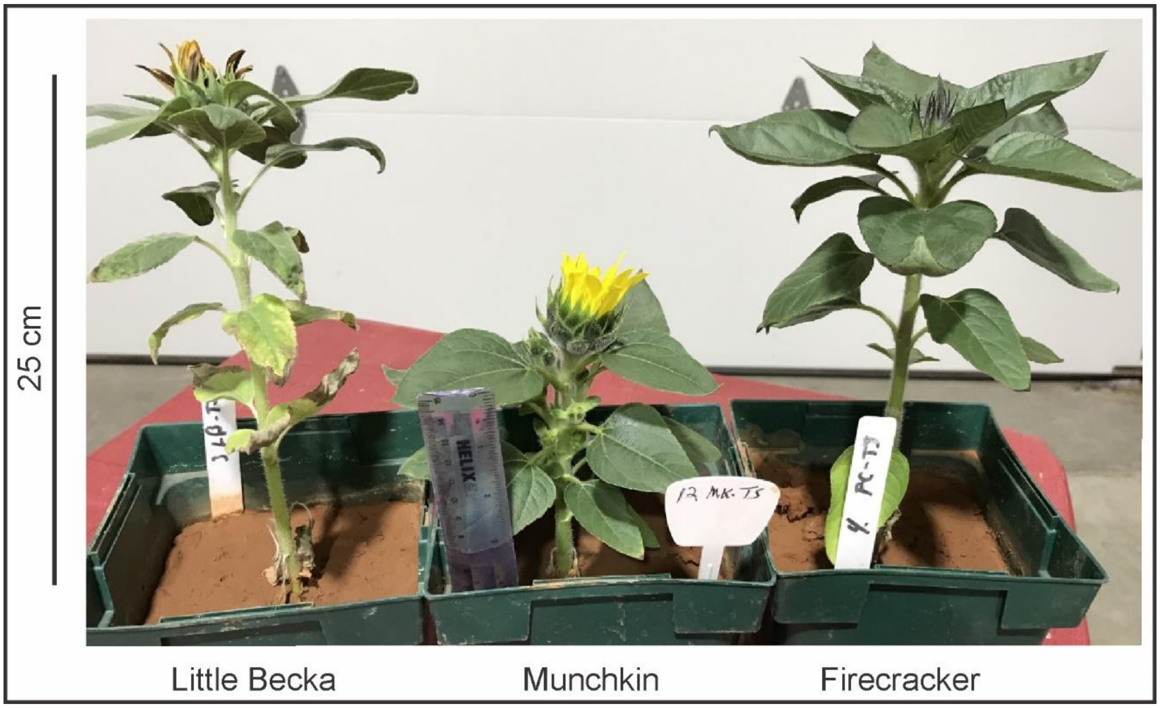

Figure 3. Little Becka, Munchkin, and Firecracker in bud/ flower at time of harvest in alkaline soil.

Munchkin is a very dwarf sunflower cultivar and this is shown in Figure 3. Firecracker and Little Becka had the largest leaf biomass. In summary, it is clear that Little Becka and Firecracker grow better and produce higher yields of HC in St. George, alkaline soil than Munchkin.

Of the 42 plants of Munchkin, Little Becka and Firecracker grown in very alkaline soil, only one Firecracker plant survived (Fig. 4). The **entire** dry weight (stem, leaves and roots) of the plant (Fig. 4) was only 0.06 g! It consisted of 4 leaves (shown in Fig. 4). One can see 3 aborted leaf scars on the stem above the 4 leaves (Fig. 4). Generally, after 2 or 4 leaves were produced, all plants died in the extreme alkaline soil. It might be noted that the natural area where the very alkaline soil occurred was devoid of any vegetation.

Extraction of this plant proved un-feasible due to extremely low HC yield.

Figure 4. Entire Firecracker sunflower plant growing in very alkaline soil. The plant (~5 cm above ground) was harvested the same day at the 12-28 cm tall plants seen in Figure 3 (above).

Soil analyses of the alkaline soils from St. George, UT revealed the alkaline (locally called 'top soil') is similar to 'very alkaline' but the 'very alkaline' soil has about 4 times as much Calcium (Ca) and Sulfur (S), Both soils are generally low in K, Mg, Na, Nitrogen, Mn, Fe, Cu, and B (Fig. 5). It is easy to see that both soils, but especially the very alkaline soil, are difficult to obtain plant growth.

SAMPLE ID	LAB NUMBER	Organic Matter		Phosphorus		Potassium	Magnesium	Calcium	Sodium	
		* % Rating	** ENR lbs/A	P1 (Weak Bray) **** * ppm	$NaHCO_3$-P (OlsenMethod) **** * ppm	K ***** * ppm	Mg *** * ppm	Ca *** * ppm	Na *** * ppm	Soil pH
alkaline	54705	0.8L	46	1 *	13M	86L	216M	2602VH	49L	7.6
very alkaline	54706	2.4M	79	7 *	4L	206L	418L	10580VH	172L	7.8

SAMPLE NUMBER	Nitrogen NO_3-N ppm	Sulfur SO_4-S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Excess Lime Rating	Soluble Salts mmhos/cm	Chloride Cl ppm
alkaline	2VL	792VH	1.2M	1VL	3VL	0.4L	0.9M	H	2.9H	
very alkaline	13M	10580VH	0.2VL	1VL	1VL	0.2VL	6.1VH	M	4.1VH	

Figure 5. Soil analyses of alkaline and very alkaline soils from St. George, UT. Only one plant survived in the 'very alkaline' soil (see Fig. 3). *Weak Bray unreliable at M or H excess lime or pH > 7.5.

Due to the unexpected CovID-19 virus pandemic that displaced the experiment from the PVHS greenhouse to the lab growth chamber, it is not statistically valid to compare the current results with the previous study on salt concentrations and sand (Johnson, Theobald and Adams 2019). However, it is worthwhile to make some qualitative estimates (Fig. 6). Munchkin plants grown in alkaline soil had much lower % HC yield (3.16), in contrast plants in high salt treatments that yielded large % HC yields. The plants growing in alkaline soil also had much lower biomass (comparable to the pure sand test), and much lower g HC/ g 10 leaves (again comparable to the sand treatment). It was thought (Johnson et al. 2020) that the sand was nutrient limiting (low in N, and organic matter) leading to low growth, and that is likely a factor for the alkaline soil.

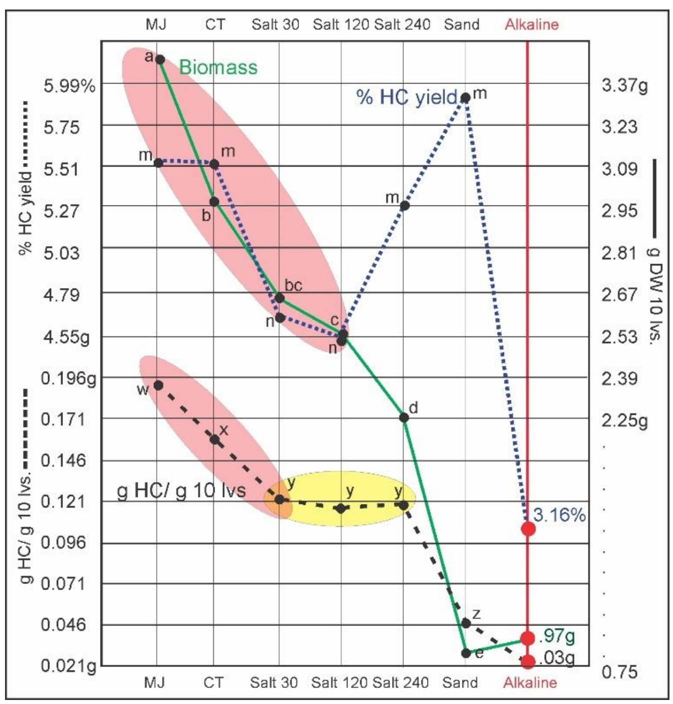

Fig. 6. Qualitative comparison of Munchkin's HC data with CT (Munchkin) in potting soil and especially in sand.

The three sunflower cultivars (Munchkin, Firecracker and Little Becka) varied in their ability to grow in alkaline, St. George soil. Munchkin was lowest in biomass, % HC yields, and g HC/ g DW 10 leaves. Little Becka was highest in % HC yields. Little Becka and Firecracker were (statistically) about equal in their biomass and g HC/ g DW 10 leaves yields. Overall, all the cultivars struggled to grow in the difficult alkaline soil with calcium levels of 2,602 ppm and sulfur 792 ppm, and being low in nitrogen and critical trace metals.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Adams, R. P. and G. J. Seiler. 1984. Whole plant utilization of sunflowers. Biomass 4: 69-80.

Adams, R. P., M. F. Balandrin, K. J. Brown, G. A. Stone and S. M. Gruel. 1986. Extraction of liquid fuels and chemical from terrestrial higher plants. Part I. Yields from a survey of 614 western United States plant taxa. Biomass 9: 255-292.

Adams, R. P. and A. K. TeBeest. 2016. The effects of gibberellic acid (GA3), Ethrel, seed soaking and pre-treatment storage temperatures on seed germination of *Helianthus annuus* and *H. petiolaris*. Phytologia 98: 213-218.

Adams, R. P., A. K. TeBeest, B. Vaverka and C. Bensch. 2016. Ontogenetic variation in hexane extractable hydrocarbons from *Helianthus annuus.* Phytologia 98: 290-297

Adams, R. P. and A. K. TeBeest. 2017. The effects of different concentrations of gibberellic acid (GA3) on seed germination of *Helianthus annuus* and *H. petiolaris* Phytologia 99: 32-35.

Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet, C. Parker and D. Thornburg. 2017a. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 1-10.

Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet and D. Thornburg. 2017b. Geographic variation in volatile leaf oils (terpenes) in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 130-138.

Adams, R. P., A. K. TeBeest, T. Meyeres and C. Bensch. 2017c. Genetic and environmental influences on the yields of hexane extractable hydrocarbons of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 186-190.

Adams, R. P. and S. T. Johnson. 2018. The effects of methyl jasmonate on the growth and yields of hydrocarbons in *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 100: 177-182.

Adams, R. P., A. K. TeBeest, S. McNulty, W. H. Holmes, J. A. Bartel, M. Corbet, C. Parker, D. Thornburg and K. Cornish. 2018a. Geographic variation in natural rubber yields in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 100: 19-27.

Adams, R. P., Matt Lavin and Gerald P. Seiler. 2018b. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers) II. Phytologia 100: 153-160.

Adams, R. P., Matt Lavin, Steve Hart, Max Licher and Walter Holmes. 2018c. Screening hydrocarbon yields of sunflowers: *Helianthus maximiliani* and *H. nuttallii* (Asteraceae). Phytologia 100: 161-166.

Johnson, S. T., S. Theobald and R. P. Adams. 2019. The effects of plant growth regulator (methyl jasmonate), salt (NaCl) stress and nutrient deficiency on biomass and hydrocarbon yields in *Helianthus annuus* cv. Munchkin (Asteraceae, Sunflowers). Phytologia 101(3): 194-199.

Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath and M. Whalen. 2010a. Natural rubber quantification in sunflower using automated solvent extractor. Indust. Crops and Prods. 31: 469-475.

Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath, J. L. Brichta and J. E. van Fleet. 2010b. Agronomic and natural rubber characteristics of sunflower as a rubber-producing plant. Indust. Crops and Prods. 31: 481-491.

Seiler, G. J., M. E. Carr and M. O. Bagby. 1991. Renewables resources from wild sunflowers (*Helianthus* spp., Asteraceae). Econ. Bot. 45: 4-15.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. New York.

Whittstock, U. and J. Gershenzon. 2002. Constitutive plant toxin and their role in defense against herbivores and pathogens. Curr. Opin. Plant Biol. 5: 300-307.