Comparison of hydrocarbon yields in four cotton accessions: regular vs. limited (induced dryland) irrigation

Robert P. Adams
Baylor University, Baylor-Utah Lab, 201 N 5500 W, Hurricane, UT 84737
robert_adams@baylor.edu

and

Mauricio Ulloa, Travis Witt and **John Burke**
USDA-ARS, PA, CSRL, Plant Stress and Germplasm Development Research,
3810 4th Street, Lubbock, TX 79415

ABSTRACT

Four Upland accessions of cotton *Gossypium hirsutum* (SA-1181, 1403, 1419, and 2269) were grown in limited irrigation and regular irrigated field conditions to compare water stress effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves). Analyses revealed a significant difference between irrigations and significant interaction between entries by irrigation or genotype by environment (GxE) for % HC yield and total HC ($p < 0.05$). However, when entries were examined in each irrigation, no significant differences were found among entries for % yield HC, and significant differences ($p = 0.03$) were found for total HC under regular irrigation while high significant differences were found under limited irrigation or induced stress/dryland field conditions for these two traits ($p < 0.001$). The average % HC yields and HC total were significant for SA-2269 compared to SA-1181, 1403, and 1419 under stress/dryland field conditions. The percent increase in HC yields (regular to limited irrigation) were: SA-1181 18.1%; SA-1403 21.5%; SA-1419 30.3% and SA-2269 42.1%. HC yields (as g HC/leaf DW) were either barely significant (SA-1181, p=0.037*) or non-significant (SA-1403, SA-1419), except for SA-2269, that was very highly significant due to the fact that the biomass was almost as large in limited as in regular irrigated plots and % HC yield was much greater than in regular irrigated plots. These results clearly show that the yields of HC in cotton are higher for these accessions when grown under water stress. In addition, there are some differential responses among the four accessions, with SA-2269 showing the greatest increase in HC yields in induced stress/dryland conditions. Published on-line www.phytologia.org *Phytologia 100(1): 1-5 (Mar 16, 2018).* ISSN 030319430.

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Cotton (*Gossypium* spp.) is a perennial shrub / tree that is grown as an annual crop in most of the world and our cultivated varieties have an unusual evolutionary history (Wendel and Grover, 2015). Even though cotton is the most important renewable natural fiber (Ulloa et al. 2007), alternative sustainable, renewable sources of petrochemicals and fuels from arid and semi-arid land as the cotton crop may be another source of income for producers. Screening 614 taxa from the western US for their hexane soluble hydrocarbon (HC) and resin (methanol soluble) yields, Adams et al. (1986) found the highest HC yielding species were from arid and semi-arid lands in the Asteraceae (11 species), Asclepiadaceae (1), Celastraceae (1), Clusiaceae (1) and Euphorbiaceae (1). The top 2% (12/614) had whole plant HC yields ranging from 10.4 to 16.4%.

Recently we reported (Adams et al. 2017a) on the yields of pentane extractable hydrocarbons (HC) from leaves of thirty USDA germplasm cotton accessions (Hinze et al. 2016), grown with supplemental underground drip irrigation at College Station, TX. We discovered % HC yields were very high in four accessions: SA-1181 12.32%; SA-1403 9.08%; SA-1419 13.23% and SA-2269 11.09% HC. Per plant HC yields varied from 0.119 to 0.178 g/ g leaf dry weight (DW). The correlation between % HC yield and average leaf DW was non-significant (0.092), suggesting that breeding for increased HC and plant biomass may be possible.

Adams et al. (2017b) examined these four high HC yielding Upland cotton (*G. hirsutum* L.) accessions (SA-1181, 1403, 1419, and 2269) grown both in field conditions and a greenhouse to compare the environmental effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves) under natural and controlled (protected) conditions. Leaf biomass was similar but higher in two field cultivated accessions. All four accessions produced higher % HC yields under field conditions, with greenhouse plants producing lower yields ranging from 20.6 to 46.0% as much HC as found in naturally field-grown plants. Total HC yields (g HC / g 10 leaves) were even lower in the greenhouse with yields being only 19.7 to 39.1% as high as from field-grown plants. Overall, the environmental component to the yield of free HC in cotton leaves was a major factor, with the genetic component contributing to less than half (46%) of the HC yield. This trend corresponds to literature reports of large induction of defense chemicals in cotton upon attack by herbivores and diseases (Chen 2008; Karban and Myers 1989; Opitz et al. 2008; Pare and Tumlinson 1997, 1998; Stipanovic et al. 1999; Turlings 1995). Ontogenetic variation in HC for SA-2269 showed HC yields in leaves remained at a constant, low level from bud to flowering, then increased rapidly as bolls matured (Adams et al. 2017b).

The purpose of this paper is to report on changes in HC production in four high HC yielding accessions of cotton (SA-1181, 1403, 1419, and 2269) grown with regular irrigation versus under limited irrigation or growing conditions to induce water stress similar to dryland stress field conditions in Lubbock, TX.

MATERIALS AND METHODS

Four accessions: (SA-1181Acala SJ-1,; 3010, SA-1403; China 86-1, SA-1419; TM 1, SA-2269) were grown at the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX under two irrigation regimes (regular and limited). Seed from each accession was planted at water field capacity and water was applied if needed until plant establishment. Irrigation regimes started after initial-squaring and continued through crop cutoff. Regular irrigation was set to 5mm/daily, and the limited irrigation or induced water stress similar to dryland stress conditions was set to 1.5 mm/daily. Each irrigation trial used a randomized complete block design with three replicated plots per accession. Irrigation was applied using sub-surface drip irrigation. Buffer or cotton plots were placed around and between each irrigation trial to ensure that one rate did not affect the plants within the other. In addition, irrigation rates were adjusted due to rainfall events to ensure differences in plant stress between the two irrigation rates, applying water only for one week for the limited irrigation trial for this experiment.

The ten (10) lowest growing mature leaves were collected from each of fifteen (15) plants for each of the four (4) accessions, in both regular irrigated and limited irrigation or dryland plots. At the time of collection, plants were at mid to completed full boll maturity, and leaves did not have insect or pest damage. Leaves were air-dried (48 hr., 45°C, to 7% moisture). Dried leaves were ground in a coffee mill (1mm). Three grams of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane. The jar was sealed and 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 was evaporated on a hot plate (70°C) in a hood. The pan with hydrocarbon extract was weighed and tared. The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. All shaker extraction yields were adjusted to oven dry wt (ODW) by a correction factor of 1.085. The soxhlet extraction correction factor was x1.14. Thus, the total correction factor (CF) utilized was 1.085 x 1.14 = 1.24.

Data were analyzed using proc glimmix with random block and block*accession, with adjust=simulate lines procedure for mean separation using the SAS statistical software (SAS Institute, Cary, NC, USA). In addition, statistical analyses (means, variance, standard deviation, standard error of mean, t-test) were performed by use of GrafPad.com and used for the figures and table reported herein. https://www.google.com/search?q=standard+deviation+calculator&oq=standard&aqs=chrome.2.69i57j69i60j35i39l2j0l2.3811j0j8&sourceid=chrome&ie=UTF-8

RESULTS

Analyses (using SAS) of interaction between GxE utilized pooled samples (all four accessions) revealed significant difference between irrigations and significant interaction between entries by irrigation or genotype by environment (GxE) for % HC yield and total HC ($p < 0.05$). Because of the GxE interaction, we separately analyzed the irrigation trials. When we examined the entries in each irrigation, no significant difference was found among entries for % yield HC, and significant difference at $p = 0.03$ was found for total HC under regular irrigation. When we examined the entries under limited irrigation or induced stress/dryland field conditions, significant difference at $p = 0.03$ were found for these two traits ($p < 0.001$). The average % HC yields and HC total were significant for SA-2269 compared to SA-1181, 1403, and 1419 under stress/dryland field conditions. In addition, high correlation was found between % HC yield and total HC ($r = 0.80$ regular and $r = 0.77$ limited irrigation) in the four accessions.

Comparison of the four accessions of cotton leaf biomass and HC yields for field grown under limited irrigation (from here on referred as dryland) and irrigated conditions, Lubbock, TX are given in Table 1. Note that none of the four accessions had significantly more biomass in irrigated than in dryland plots using the t test. This is because the summer of 2017 was unusually wet in Lubbock, leading to much smaller effects than normally experienced.

For detailed comparisons for each genotype, t-tests were run on each accession (15 plants under regular irrigation and 15 plants under reduced irrigation). The reader should note that the foregoing results differ a little from the above SAS analysis due to the different statistical models used. Using t-tests, the % HC yields were significant for SA-1181, and very highly significant for SA-1403, 1419 and 2269 (Table 1). The percent increase in HC yields (irrigated to dryland) were: SA-1181 18.1%; SA-1403 21.5%; SA-1419 30.3% and SA-2269 42.1%. The % HC yields were least affected by reduced irrigation in SA-1181, and the % HC was most increased by drought in SA-2269. Based on this factor alone (% HC yield), one would prefer SA-2269 if the crop were grown dryland in semi-arid lands.

Table 1. Comparison of four accessions of cotton leaf biomass and HC yields for field grown under dryland and irrigated conditions, Lubbock, TX. Significance: ns = non-significant; * = *p* 0.05; ** = *p* 0.01; *** = *p* 0.001.

	DW for 10 lvs/plant, +/- 2 std err.			% HC yield, +/- 2 std err.			HC g/ leaf DW, +/- 2 std err.		
Accession	dryland	irrigated	t-test	dryland	irrigated	t-test	dryland	irrigated	t-test
SA-1181	9.42 g +/-1.52	8.98 g +/-0.79	*p*= 0.253 ns	4.08 % +/- 0.30	3.34 % +/- 0.42	*p*= .0042 **	0.0381g +/-0.006	0.0305 g +/-0.005	*p*= 0.037 *
SA-1403	7.87 g +/-0.60	8.40 g +/-1.24	*p*= 0.225 ns	3.91 % +/-0.26	3.07 % +/-0.20	*p*= .0005 ***	0.0312g +/-0.003	0.0264 g +/-0.005	*p*= 0.053 ns
SA-1419	6.99 g +/-0.79	8.01 g +/-0.92	*p*= .05 *	4.19 % +/-0.84	2.92 % +/-0.39	*p*= .0005 ***	0.0293g +/-.004	0.0239 g +/-.004	*p*= 0.506 ns
SA-2269	8.99 g +/-0.59	9.63 g +/-0.63	*p*= 0.762 ns	5.35 % +/- 0.35	3.10 % +/- 0.30	*p*= .00005 ***	0.0487 g +/-0.005	0.0300 g +/-0.004	*p*= 0.0005 ***

The lack of variation in biomass (g DW 10 leaves) is shown in Figure 1. There is only one significant difference between dryland and irrigated in any of the accessions using the t-test.

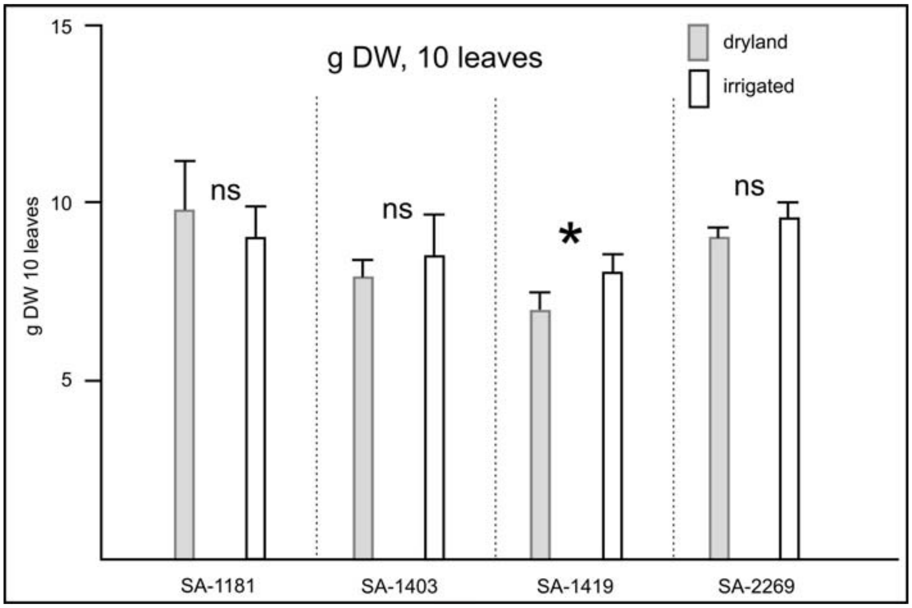

Figure 1. Bar graph of biomass (as g DW 10 leaves) for dryland and irrigated cotton accessions.

Figure 2 shows that each of the accessions had highly or very highly significantly larger % HC yields in dryland conditions. By far the largest increase was found with SA-1419 and SA-2269 having 30.3 and 42.1% higher % HC yields in dryland than in irrigated conditions (Fig. 2).

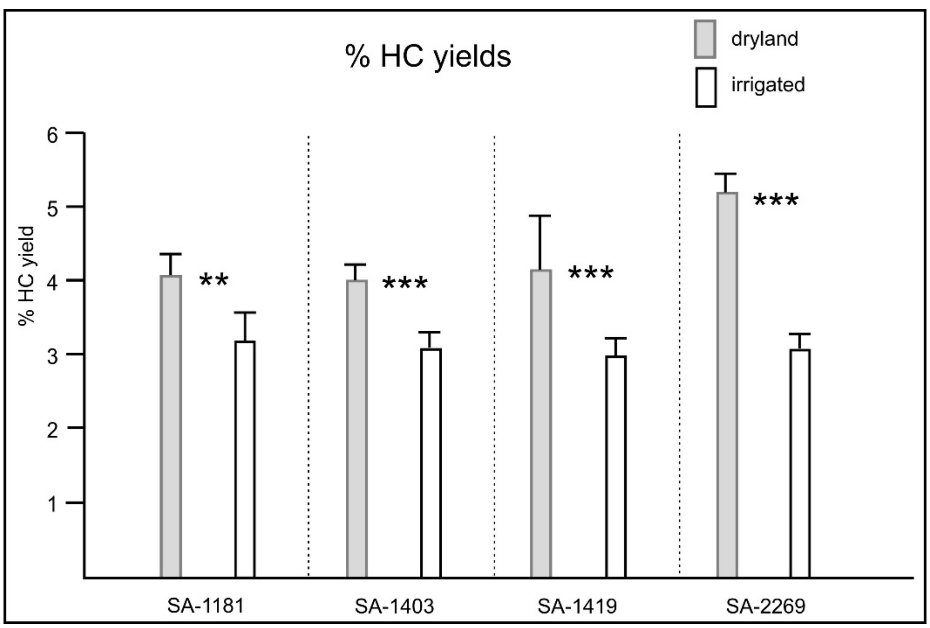

Figure 2. Bar graph of % HC yields for dryland and irrigated cotton accessions.

The HC yields (as g HC/leaf DW) were not significant for SA-1403 and SA-1419 under irrigated but with significant values for SA-1181 and highly significant differences under dryland conditions for SA-2269 due to the fact that the biomass was almost as large in dryland as in irrigated and % HC yield was also very much greater than in irrigated (Table 1.), thus, the product (biomass wt x % HC) was significantly greater (Table 1). Again, based on this factor alone (total g HC), one would prefer SA-2269 if the crop were grown dryland in semi-arid lands. Figure 3 shows all the accessions have larger HC yields (as g HC/ leaf DW), except SA-1403 and SA-1419 were not significant. Yields for SA-1181 and

SA-2269 were significant (*) and very highly significant (***). For SA-2269, the dryland g HC/ leaf DWyield was 38.4% greater than from irrigated plants.

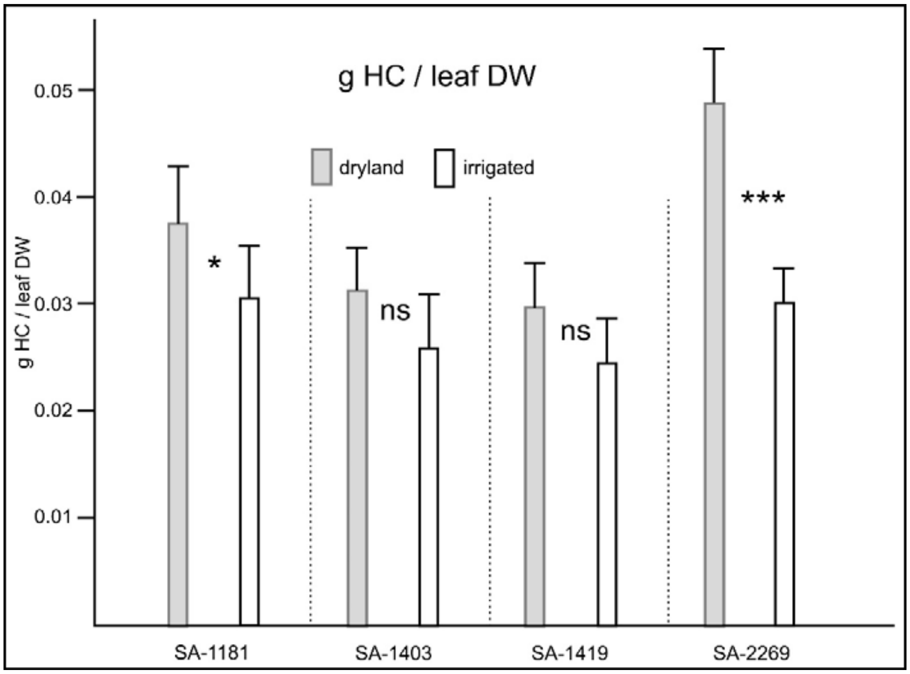

Figure 3. Bar graph of g HC/ leaf DW yields for dryland and irrigated cotton accessions.

Some of these % HC yield increases in dryland are actually moderate compared to the situation that occurred in College Station, TX, where, in the summer of 2016, these four accessions had much higher % HC yields: SA-1181 12.32%; SA-1403 9.08%; SA-1419 13.23% and SA-2269 11.09%. These larger amounts in the 2016 % HC yields appear to be due to some other factor than drought as they were grown on subsurface drip irrigation, in sandy soil, with an annual rainfall of 40" (Adams, et al. 2017a). It seems likely that these four accessions were damaged by insects or some disease and that led to the induction of defense chemicals, giving these atypically high yields in 2016 test plot.

The results from this study clearly show that the yields of HC are higher for these accessions when grown under water stress. In addition, there are some differential responses among the four accessions, with SA-2269 showing the greatest increase in HC yields in dryland conditions. Even though cotton is the most important renewable natural fiber, alternative sustainable, renewable sources of petrochemicals and fuels from arid and semi-arid land as the cotton crop may be another source of income for producers. In addition, in the Texas High Plains often-extended periods between rainfall events can lead to a reduction in the yield and fiber quality of cotton (*G. hirsutum*). Identifying morphological or physiological traits associated with plant water stress or drought could allow researchers or breeders to select/identify more efficiently for superior cotton with improved tolerance or sensitivity to drought. Further research (especially under normal, very dry summers in Lubbock) is needed to more fully understand these trends.

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LITERATURE CITED

Adams, R. P., A. K. TeBeest, J. Frelichowski, L. L. Hinze, R. G. Percy, M. Ulloa and J. Burke. 2017a. Survey of Cotton (*Gossypium* sp.) for non-polar, extractable hydrocarbons for use as petrochemical feedstocks. Phytologia 99: 54-61.

Adams, R. P., A. K. TeBeest, M. Ulloa, T., J. Burke and J. Frelichowski and L. L. Hinze. 2017b. Comparison of hydrocarbon yields in cotton from field grown vs. greenhouse grown plants. Phytologia 99(3): 200-207.

Chen, M-S. 2008. Inducible direct plant defenses against insect herbivores: A review. Insect Science 15: 101-114.

Hinze, L.L., E. Gazave, M.A. Gore, D.D. Fang, B.E. Scheffler, J.Z. Yu, D.C. Jones, J. Frelichowski and R.G. Percy. 2016. Genetic diversity of the two commercial tetraploid cotton species in the *Gossypium* Diversity Reference Set. Journal of Heredity 107: 274-286.

Karban, R. and J. H. Myers. 1989. Induced plant responses to herbivory. Ann. Rev. Ecol. Syst. 20: 331-348.

Opitz, S., G. Kunert and J. Gershenzon. 2008. Increased terpenoid accumulation in cotton (*Gossypium hirsutum*) foliage is a general wound response. J. Chem. Ecol. 34: 508-522.

Pare, P. W. and J. H. Tumlinson. 1997. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol. 114: 1161-1167.

Pare, P. W. and J. H. Tumlinson. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. Phytochemistry 47: 521-526.

Stipanovic, R. D., A. A. Bell and C. R. Benedict. 1999. Cotton pest resistance: The role of pigment gland constituents. pp. 211-220. in: Biologically active natural products: Agrochemicals. H. G. Cutler and S. J. Cutler, eds., CRC press, Boca Raton, FL.

Turlings, T. C. J., J. H. Loughrin, P. J. McCall, U. S. R. Rose, W. J. Lewis and J. H. Tumulinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Natl. Acad. Sci. 92: 4169-4174.

Ulloa, M., C. Brubaker, and P. Chee. 2007 Cotton. In: Kole, C. (Ed.) Genome Mapping & Molecular Breeding. Vol. 6: Technical Crops. Springer, Berlin, Heidelberg, New York. 1-49.

Wendel, J. F. and C. E. Grover. 2015. Taxonomy and evolution of the cotton genus, *Gossypium*. In: Cotton, 2nd ed., D. D. Fang and R. G. Percy, eds., Agronomy Monograph 57.