

**Comparison of hydrocarbon yields in cotton from field grown vs. greenhouse grown plants.****Robert P. Adams and Amy K. TeBeest**

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**ABSTRACT**

Four accession of cotton (SA-1181, 1403, 1419, and 2269) were 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 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. The same pattern has been found in sunflowers and is discussed in regards to cotton. 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. Published on-line [www.phytologia.org](http://www.phytologia.org) *Phytologia* 99(3): 200-207 (Aug. 8, 2017). ISSN 030319430.

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Many plant species protect themselves from herbivory by a response to an attack (see Karban and Myers, 1989 for a review). It appears that early research on plant defensive chemicals focused on constitutive (or stored) chemicals such as terpenoids, tannins and aromatic metabolic compounds derived from the shikimic acid pathway (Pare and Tumlinson, 1998). But, more recently, greater focus has been on inducible plant defenses (Chen 2008; Pare and Tumlinson, 1997, 1998; Turlings, et al. 1995). Turlings et al. (1995) published a seminal paper entitled "How caterpillar-damaged plants protect themselves by attracting parasitic wasps". They showed that plants injured by herbivores emit chemical signals that attract and guide the herbivores' natural enemies to the damaged plants. Thus, indirectly, injured plants send out a "SOS" signal for help against herbivores. Pare and Tumlinson (1997) nicely documented this phenomenon in a series of experiments on cotton using beet army worms and mechanical damage to leaves.

Chen (2008) discusses that some constitutive chemicals may be increased to even higher levels after insect attack. The present research (herein) is concerned with total extractable hydrocarbons for alternative fuels and chemical feedstocks from cotton leaves.

In a seminal paper, Stipanovic, Bell and Benedict (1999) reviewed the defensive role of pigment gland constituents in cotton. Cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivatives, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed that these gland constituents can be rapidly synthesized in response to pathogens.

Opitz, Kunert and Gershenzon (2008) examined the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They found that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments. In addition, they reported that herbivory or mechanical damage in older leaves led to terpenoid increases in younger leaves. Higher terpenoid yields were achieved by two methods: 1. increased filling of existing glands and 2. the production of additional glands. The composition of the terpenoid mixture did not significantly differ in response to herbivore, mechanical damage or jasmonic acid treatments.

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 with 11.34, 12.32, 13.23 and 13.73 % HC. Per plant HC yields varied from 0.023 to 0.172 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.

In addition, Adams et al. (2017a) conducted an ontogenetic study of a commercial cotton cultivar, (FiberMax 1320), grown under dryland conditions. They reported the dry weight of leaves reached a maximum at the 1st flower stage, and then declined as bolls opened. However, % pentane soluble hydrocarbon yields continued to increase throughout the growing season (due to the decline of leaf DW). It seems likely that as the bolls mature and seed are filled, carbohydrates from the leaves are catabolized and sugars are transported to the bolls for utilization. Per plant HC yields increased from square bud stage to 1st flower, remained constant until 1st boll set, then declined at 1st boll-opened stage. This seems to imply that most of the HC are not catabolized and converted to useable metabolites for filling bolls and seeds.

The evolution of modern cotton (*Gossypium* spp.) encompasses an improbable series of events that involved transoceanic, long-distance dispersal with hybridization involving two diploids, one from the Old World and one from the New World, forming the modern cultivated allo-tetraploid, *G. hirsutum* (Wendel and Grover, 2015).

Although there are several papers on the conversion of cotton field stubble to liquid fuels (see Putun, 2010; Putuan et al., 2006; Akhtar and Amin, 2011 and references therein), there are no reports on the environmental versus genetic nature of the production of total extractable HC in cotton.

The purpose of this paper is to report on changes in HC production in field cultivated cotton compared with cotton grown in a greenhouse. In addition, data is reported on ontogenetic variation in HC production in cotton accession SA-2269.

## MATERIALS AND METHODS

### Plant Materials:

#### *Commercial, cultivated cotton*

FiberMax 1320, dryland, dark, loam soil, JP TeBeest Farm, 36° 25' 0.6" N, 101° 32' 17.3" W, 3258 ft., Oslo, TX, avg. annual rainfall, 19.3". The eight (8) lowest growing, non-yellowed mature leaves were collected at random from each of 10 cotton plants, at square bud, 1st open flower, 1st boll, and 1st boll completely opened stages. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

#### *HC yields of 4 high yielding HC cotton accessions grown in a greenhouse*

Four accessions (Acala SJ-1,SA-1181; 3010, SA-1403; China 86-1, SA-1419; TM 1, SA-2269) were grown the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX. Acala SJ-1,SA-1181; 3010, SA-1403; China 86-1, SA-1419; TM 1, SA-2269 cotton seeds were planted into 27 cm diameter pots containing Sunshine Mix #1 soil (Sun Gro Horticulture Distributors Inc., Bellevue, WA). Three seeds were planted per pot and pots were placed on benches in a greenhouse set to provide a 31/27°C day/night cycle. Plants were thinned to one plant per pot and grown throughout the experiment. 430 W high-pressure sodium lights (P. L. Light Systems, Beamsville, ON Canada) were used to maintain a 16/8 h photoperiod. Nutrients were maintained by daily application with Peters Excel fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) through the automated watering system.

For comparison, the HC yields and leaf DW from the greenhouse study were compared with data from these four accessions (Acala SJ-1,SA-1181; 3010, SA-1403; China 86-1, SA-1419; and TM 1, SA-2269) that were cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, during the summer of 2016 (see Materials and Methods, Adams et al. 2017).

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 pentane. The jar was sealed, then placed on an orbital shaker for 18 hr. The pentane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan, and the pentane was evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-pentane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the pentane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with pentane for 8 hrs. All shaker extraction yields were adjusted to oven dry wt (ODW) by a correction factor (CF) of 1.085. For the cultivated cotton from Oslo, TX, the shaker yields were corrected by the increased soxhlet extraction efficiency (CF = x1.56). For the Lubbock accessions, the soxhlet CF was x1.31 and for the accessions grown at College Station, the soxhlet CF was x1.69.

Statistical analyses (means, variance, standard deviation, standard error of mean) were performed by use of EasyCalculation (<https://www.easycalculation.com/statistics/standard-deviation.php>)

## RESULTS

Biomass and hydrocarbon (HC) yields in greenhouse vs. field grown data for four accessions (Table 1) shows that the leaf biomass is similar, but HC yields (as % HC yields) are considerably lower in greenhouse conditions.

Table 1. Comparison of leaf biomass and HC yields for greenhouse versus field grown cotton.

Accession	Greenhouse grown, Lubbock, TX bolls maturing			Field grown, College Station, TX flowering and with bolls		
	DW for 10 lvs/plant, 2 std err.	% HC yield, 2 std err.	HC g/ 10 lvs DW, 2 std err.	DW for 10 lvs/plant	% HC yield	HC g/ 10 lvs DW
SA-1181	9.86 g 1.543	4.25 %, 0.355	0.419 g, 0.0552	9.62 g	12.32 %	1.19 g
SA-1403	11.38 g 1.230	4.18 % 0.336	0.476 g 0.0420	14.63 g	9.08 %	1.33 g
SA-1419	11.50 g 3.243	2.73 % 0.600	0.340 g 0.157	13.10 g	13.23 %	1.73 g
SA-2269	11.93 g, 0.966	4.50%, 0.543	0.537 g, 0.0782	12.44 g	11.09 %	1.38 g

Similar leaf biomass was observed for SA-1181 and SA-2269, but larger leaf biomass was obtained from field grown SA-1403 and SA-1419 (Fig. 1) than from greenhouse grown plants.

In contrast, large differences were found in the % yields of HC (Table 1, Fig. 2). The ratio of % yields in greenhouse / field grown varies as: SA-1181 - 34.5%; SA-1403 - 46.0%; SA-1419 - 20.6%; and SA-2269 - 40.5%. In spite of the robust growth achieved in the greenhouse, these accessions yielded only 20.6% to 46.0% as much as when cotton was field cultivated and exposed to natural challenges in the environment.

The lower % yield of SA-1419 (Fig. 2) in protected conditions (i.e., greenhouse) seems to imply the genotype is particularly affected by insects, diseases, water stress, etc. that apparently induced increased HC production in the field (13.23% vs. 2.73% greenhouse). This suggests that higher HC yields might be induced by applying stresses, and some may be inducible with the right growth regulators, etc. But, it also is a note of caution that the farmer may be at risk of producing low HC yields when 'ideal' growing conditions occur.

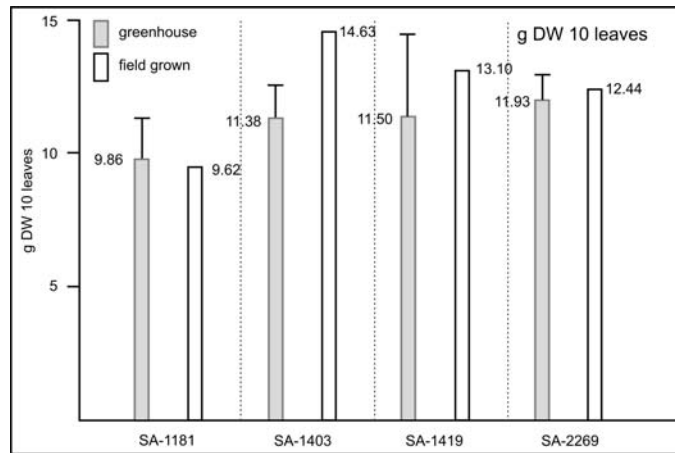


Fig. 1. Comparison of g DW of 10 leaves for greenhouse vs. field grown plants for four cotton accessions (at College Station, 2016). Note: standard error of the mean could not be obtained for the 2016 data, as leaves from plants were combined for each accession)

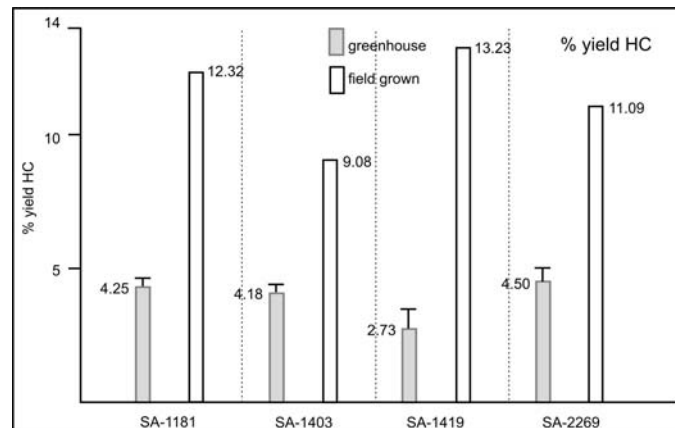


Fig. 2. Comparison of % yield of HC from greenhouse vs. field grown plants (at College Station, TX, 2016).

The trend seen for the g HC / 10 leaves data (Table 1, Fig. 3) is very similar to that seen for the % HC yields (Table 1, Fig. 2). Again SA-1403 was very low in greenhouse plants (only 19.7% as much as field grown). The g HC / 10 leaves yields from greenhouse / field grown varies from 19.7% to 39.1%. As with the % yield data, total harvestable HC might be greatly increased by the application of growth regulators (cf. methyl jasmonate, salicylic acid, etc.) or other agents that induce the synthesis of HC in leaves.

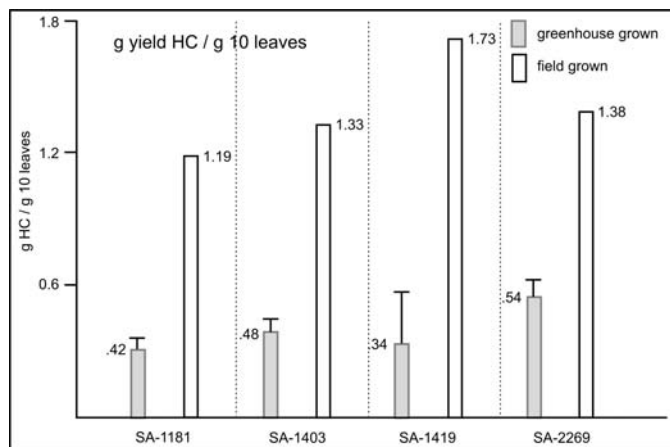


Fig 3. Comparison of g HC / 10 leaves from greenhouse vs. field grown plants (at College Station, 2016).

This same trend of lower HC yields was reported in greenhouse reared sunflowers (Adams et al. 2017b). Leaves and seeds from the same plant were collected from native *H. annuus* from Gruver, TX (GT), Lake Tanglewood, TX (LT) and Salt Lake City, UT (SLC) when the flowers had 10% to 30% disk flowers present. The seeds from each population were germinated and plants grown in the greenhouse at Oklahoma Panhandle State University, Goodwell, OK. The greenhouse grown progeny from the three natural populations had much less leaf biomass (Fig. 4, upper).

Just as seen for cotton, % yield HC was much lower in the greenhouse grown progeny (Fig. 4, center): GT - 45.6%; LT - 55.6%; SLC 78.3%. The SLC population appears to be much less affected by local environmental factors, than the populations at GT and LT.

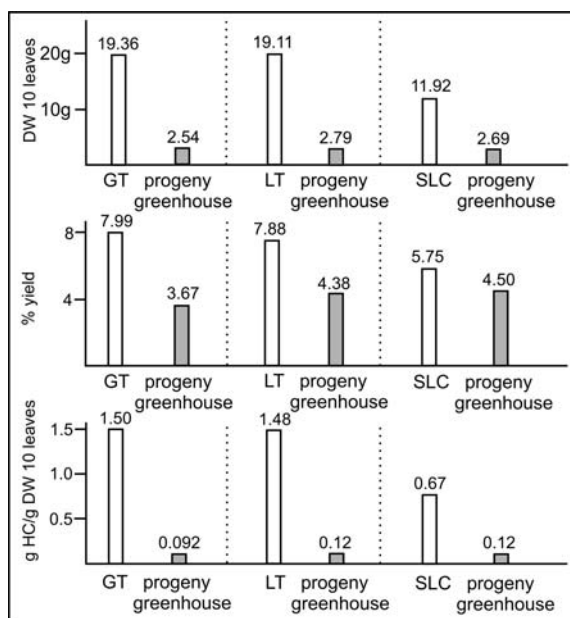


Fig. 4. Comparison of natural sunflowers versus greenhouse grown progeny (Adams et al. 2017b)

The g HC / g DW 10 leaves is similar to the % yield data (Fig. 4. lower), but with a more extreme reduction of HC (i.e, greenhouse / natural = GT - 6.1%; LT - 8.1%; SLC - 17.9%).

So, for at least two genera (*Gossypium* and *Helianthus*), there is a much reduced production of leaf HC when plants are greenhouse grown and removed from naturally occurring plant stresses that induce defense chemicals.

In addition to a study of the effects of greenhouse growth versus natural environment growth, it is of interest to examine ontogenetic variation for the production of HC in the leaves of cotton. Two cotton accessions: SA-2269 (greenhouse grown) and FiberMax 1320 (field grown, see Adams et al. 2017b) were sampled at several growth stages: bud, flowering, bolls maturing and, for FiberMax 1320, bolls open. Comparison of SA-2269 and FiberMax 1320 for leaf biomass, % HC yields and g HC / 10 leaves is presented in Table 2 and Figure 5.

Table 2 Ontogenetic variation in pentane soluble hydrocarbon (HC) yields from four cotton accessions grown at the USDA greenhouse, Lubbock, TX compared to HC yields from FiberMax 1320 field grown at Oslo, TX (Adams et al. 2017b).

Accession, collection growth stage	DW for 10 lvs/ plant, 2 std err.	% HC yield, 2 std err.	Range of yields (%)	HC g/ 10 lvs DW, 2 std err.
SA-2269, Greenhouse, Lubbock, TX, bud stage	5.79 g, 0.718	2.91%, 0.336	(2.20-3.93%)	0.163 g, 0.0419
SA-2269, Greenhouse, Lubbock, TX, flowering with some small bolls	11.08 g, 1.228	2.98%, 0.270	(2.32-3.69)	0.335 g, 0.0596
SA-2269, Greenhouse, Lubbock, TX, bolls maturing	11.93 g, 0.966	4.50%, 0.543	(3.22, 5.63)	0.538 g, 0.0782
FiberMax 1320, Oslo, TX square bud	6.85 g, 0.64	4.05%, 0.30	(3.31 - 4.56)	0.275 g, 0.032
FiberMax 1320, Oslo, TX 1st flower	9.53 g, 0.78	6.05%, 0.70	(4.78 - 7.84)	0.564 g, 0.106
FiberMax 1320, Oslo, TX 1st boll set	7.86 g, 0.72	6.99%, 0.62	(4.95 - 8.28)	0.550 g, 0.068
FiberMax 1320, Oslo, TX bolls open, seeds maturing	5.54 g, 0.57	8.02%, 0.50	(6.65 - 8.90)	0.474 g, 0.054

Both SA-2269 and FiberMax 1320 increase their leaf biomass from bud to flowering stages (Table 2, Fig. 5, upper). However, SA-2269 continued to increase leaf biomass to the bolls stage (Table 2, Fig. 5, upper). It should be noted that FiberMax 1320 was sprayed with a growth regulator between flowering and boll set, whereas the plants grown in the Lubbock greenhouse were not sprayed with a growth regulator.

The % HC yields for both SA-2269 and FiberMax 1320 continued to increase with maturity (Table 2, Fig. 5, middle). Whereas, FiberMax 1320 had a linear increase, SA-2269 yields were constant from bud to flowering, and then showed a large increase from flowering to bolls (Fig. 5, middle).

Due to the interaction of leaf biomass and % HC yields, FiberMax 1320 and SA-2269 had quite different patterns in their g HC / g 10 leaves data (Fig. 5, lower). FiberMax 1320 reached a maximum at flowering with a plateau from flowering to bolls, whereas SA-2269 had a linear increase from bud to flowering to bolls maturing (Fig. 5, lower). The curve for FiberMax 1320 should be viewed with caution, because it was sprayed with a growth regulator between flowering and boll set.

It is interesting to note that the natural populations of sunflowers with the highest % HC (Gruver, TX and Lake Tanglewood (Amarillo, TX) are in high wind areas and that Karban and Myers (1989) suggested that wind may maintain a high level of defensive chemicals. No appreciable amount of wind was present in either the cotton or sunflower greenhouse environments. In addition, the greenhouse plants were not significantly damaged by insects, except white flies that did minimal leaf damage.

These data on HC yields with maturity are encouraging, yet, illustrate the need for more detailed research to more clearly elucidate the changes in HC production in cotton in natural versus greenhouse (controlled) environments. Additional research is ongoing to further investigate the leaf biomass, % HC yields and g HC / leaves under stress conditions such different irrigation regimes (well- and limited irrigation) under a drip surface system and different developmental stages.

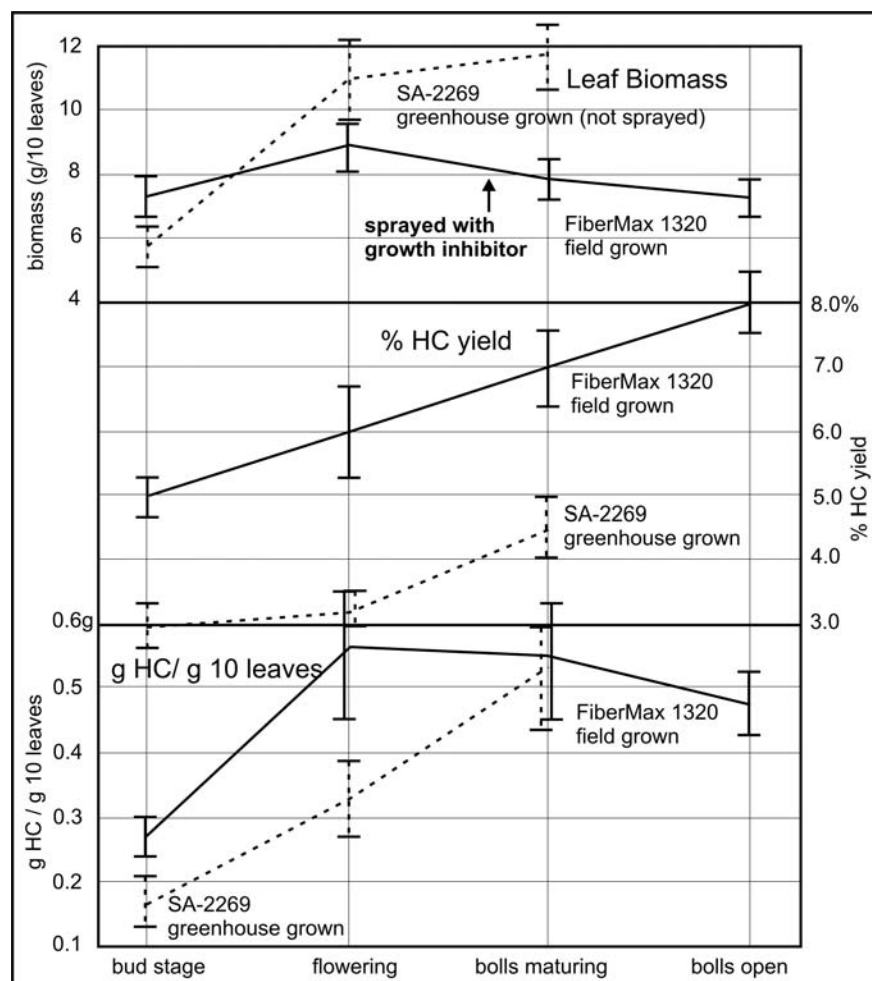


Fig. 5. Comparisons of SA-2269 and FiberMax 1320 for their leaf biomass, % HC yields, and g HC/ 10 leaves at various stages of maturity.

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