Survey of cotton (*Gossypium* sp.) for non-polar, extractable hydrocarbons for use as petrochemicals and liquid fuels

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ABSTRACT

An ontogenetic study of a commercial cotton cultivar (FiberMax 1320), grown dryland, revealed that the dry weight (DW) of leaves reached a maximum at the 1st flower stage, and then declined as bolls opened. However, % pentane soluble hydrocarbon (HC) yield 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. A survey of arid land cotton accessions, grown under limited irrigation or similar to dryland at Lubbock, TX, found % HC yield ranged from a low of 2.88% to highs of 5.78 and 5.54% . Per plant HC yields ranged from 0.017 to 0.043 g/ g leaf DW . Correlation between % HC yield and avg. leaf DW was non-significant (-0.103). A survey of USDA germplasm cotton accessions, grown with supplemental underground drip irrigation to achieve best yields germinated by irrigation, thence grown dryland at College Station, TX, found % HC yields were very high, with four accessions yielding 11.34, 12.32, 13.23 and 13.73%. Per plant HC yields varied from 0.023 to 0.172 g/ g leaf DW. Hopi had a high % HC yield (10.03%), but it was the lowest per plant yield (0.023 g/ g leaf DW). In contrast, China 86-1 with the second highest % HC yield (13.23%) was the highest per plant yield (0.172 g). The correlation between % HC yield and avg. leaf DW was non-significant (0.092). Thus, as seen in the arid land accessions, it appears that one might breed for both % HC yield and leaf DW in cotton. Published on-line www.phytologia.org *Phytologia 99(1): 54-61 (Jan 19, 2017)*. ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* sp., yields of pentane extractable leaf hydrocarbons, petrochemicals, liquid fuels.

There is a revived interest in sustainable, renewable sources of petrochemicals and fuels from arid and semi-arid land crops with the uncertainty of sustained crude oil production in the world. Adams et al. (1986) screened 614 taxa from the western US for their hexane soluble hydrocarbon (HC) and resin (methanol soluble) yields. They 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, Adams et al. (2017) surveyed native and cultivated sunflowers for their yields of leaf HC for use as a potential semi-arid land crop and found high yielding (pentane extractable HC) plants. The top 2% had HC yields (ex leaves) ranging from 10.9 to 12.6% (Fig. 1), with the top 5% ranging from 8.7% to 12.6%.

A preliminary analysis of the leaf HC yields from six locally cultivated cotton plants found a HC yield of 7.94% in one plant. In comparison, HC yields from our locally cultivated commercial sunflowers ranged from 2.75 to 3.85%, as we expected, in a crop that has been extensively selected for seed production that leads to an inadvertent selection against the production of protective phytochemicals in the leaves.

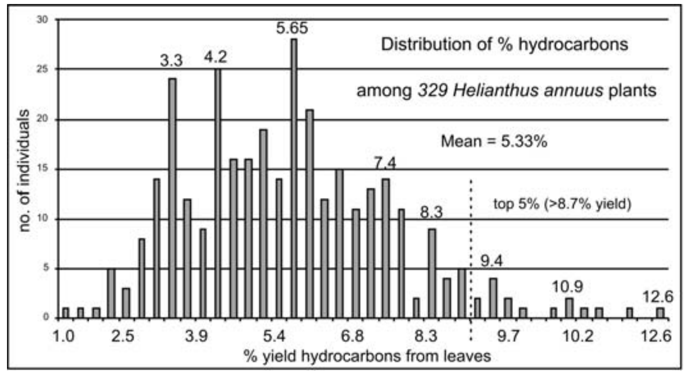

Figure 1. Frequency distribution of HC yields for 329 *H. annuus* plants (from Adams et al., 2017).

A comparison between sunflowers and cotton characteristics shows considerable differences:

characteristics	sunflowers (commercial)	cotton (commercial)
annual/ perennial	annual	perennial (but grown as an annual)
habit	herbaceous	woody
flowering	natural, 1 flower head/plant (natural: many heads/plant)	induced by growth regulators or drought, many flowers/plant (depending on photoperiod)
leaf life	lower leaves yellow and die	generally defoliate for harvest
natural habitat	temperate, North America	dry tropics (or dry sub-tropics)
origin	from *H. annuus*, North America	complex genetics from taxa from around the world (Wendel and Grover, 2015).

Annual sunflowers, herbaceous plants, live only to reproduce (by seed), whereas cotton, a woody, perennial, having evolved with a dry season to induce flowering and seed formation, has adapted to a long lifetime, in which annual seed production is not critical for short-term survival. However, maintaining plant defensive chemicals and storing energy metabolites for cotton to survive the dry season are important.

The evolution of modern cotton (*Gossypium* sp.) 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* (the reader is urged to read the informative account by 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 appear to be no surveys of the yields of non-polar HC extractables in cotton.

As a part of our research on the investigation of contemporary crops for alternative, renewable sources of petrochemical feedstocks and fuels, the present paper reports on the yields of HC from cotton (*Gossypium* sp.) cultivars and accessions.

MATERIALS AND METHODS

Plant Materials:

Ontogenetic variation in HC yields study:

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 30 cotton accessions representing photoperiodic and non-photoperiodic forms of two species:

Cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, 30 37' 5.00" N, 96 21' 50" W, 354 ft., subsurface drip irrigation, sandy soil, annual rainfall 40". The lowest growing, non-yellowed, mature leaf was collected at random, from each of 4-5cotton plants and bulked for an accession sample. Different accessions varied in growth stage from square bud, 1st flower, and 1st boll as the accessions were being grown for seed production. These accessions represent both photoperiodic and non-photoperiodic types as well as obsolete cultivars within the two commercial tetraploid cotton species, *G. hirsutum* and *G. barbadense*. These accessions were collected worldwide and are maintained by the USDA National Cotton Germplasm Collection.

HC yields 21 cotton accessions grown for drought testing:

Cultivated at the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX, 33 35' 36.3" N, 101 54' 4.2" W, 3243 ft., light, sandy soil, avg. annual rainfall 19.2". The lowest growing, non-yellowed, mature leaf was collected at random, from each of 10 cotton plants and bulked for an accession sample. Different accessions varied in growth stage from square bud to 1st flower. Some supplemental water was applied during the growing season to attain germination and limited growth to reflect plant stress responses, similar to dryland production, otherwise the plants were watered only by natural rainfall. These accessions represent a diverse pool of *G. hirsutum* germplasm with different genetic backgrounds from the USDA National Cotton Germplasm Collection.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml pentane, the jar 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 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 corrected to oven dry wt. (ODW) multiplication of 1.085. For the cultivated TeBeest cotton, the shaker yields were corrected by the increased soxhlet extraction efficiency (CF = x1.56). For the arid land accessions, the soxhlet CF was x1.31 and for the accessions grown at College Station, the soxhlet CF was x1.69.

RESULTS

Ontogenetic variation in HC yields in FiberMax 1320, grown dryland, are given in Table 1. Notice (Fig. 2) that the DW of 8 leaves (lvs) (from each plant) reach a maximum at the 1st flower stage, and then declined. However, % HC yield 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 metabolized into sugars that are transported to the bolls for utilization. Non-polar hydrocarbons such as waxes, terpene hydrocarbons, alkanes, alkenes, etc. are thought to be largely inert and not subject to catabolism. Notice that non-polar hydrocarbons (HC, as g DW/ 8 leaves) increased from square bud stage to 1st flower, remained constant until 1st boll-set, then declined at 1st boll-opened stage (Fig. 2). This seems to imply that most (~80% 0.355 g/0.440 g, Table 1) of the HC are not catabolized and converted to sugars or other metabolites that might be utilized for during the maturation of the bolls and seeds. Approximately ~80% of the non-polar hydrocarbons remain in the leaves (at least through the boll-opening stage (additional research is in planned to further examine the fate of non-polar HC).

Table 1. Ontogenetic variation in pentane soluble hydrocarbon (HC) yields in FiberMax 1320, grown dryland using eight leaves (lvs) per plants and dry weight (DW) of leaves.

collection growth stage	DW for 8 lvs/plant, std err. mean	% HC yield, std err. mean	Range of yields(%)	HC g/ 8 lvs DW, std err. mean
14949 Cotton, cult Oslo, square bud stage	5.49 g, 0.32	4.05%, 0.15	(3.31 - 4.56)	0.222 g, 0.016
14949 Cotton, cult Oslo, 1st flower stage	7.46 g, 0.34	6.05%, 0.35	(4.78 - 7.84)	0.451 g, 0.053
14949 Cotton, cult Oslo, 1st boll set	6.29 g, 0.36	6.99%, 0.31	(4.95 - 8.28)	0.440 g, 0.034
14949 Cotton, cult Oslo, 1st boll open, seeds maturing	4.43 g, 0.286	8.02%, 0.25	(6.65 - 8.90)	0.355 g, 0.027

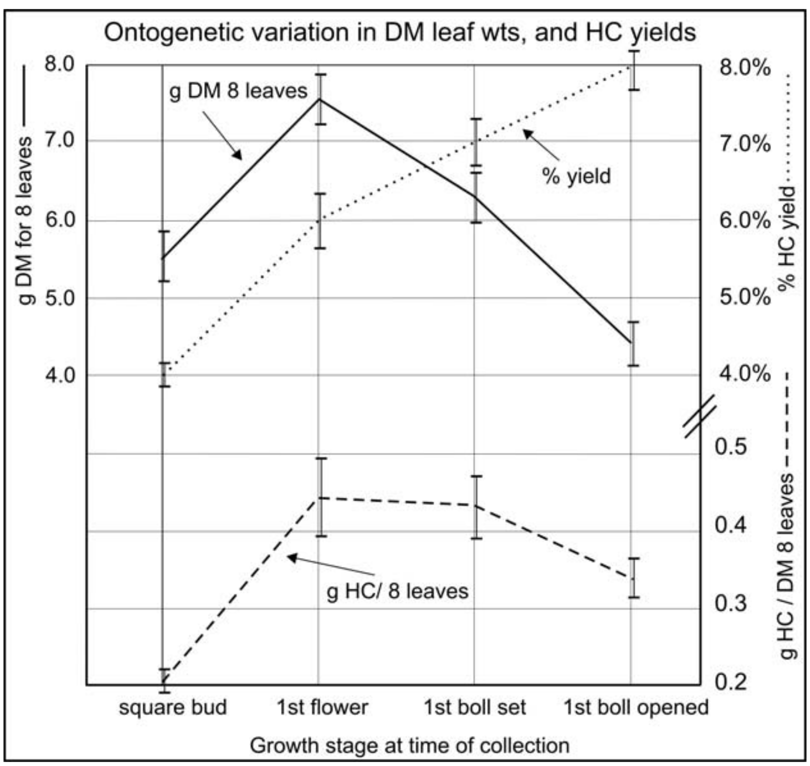

Figure 2. Ontogenetic variation in HC yields (as % HC yield and g HC/g dry leaves) in FiberMax 1320.

The survey of arid land cotton accessions growing dryland at Lubbock, TX revealed (Table 2) that % HC yield ranged from a low of 2.88% (14972, 16TXLWSA057) to highs of 5.78% (14961, 16TXLWSA039) and 5.54% (14964, 16TXLWSA043). Yields based on g HC/ g leaf DW ranged from 0.017 (14971, 16TXLWSA056) to 0.043 (14961, 16TXLWSA039 and 14965, 16TXLWSA047).

The correlation between % HC yield and avg. leaf DW was non-significant (*r* = -0.103). Thus, one might be able to breed for increases (up to some point) in both % HC yield and leaf DW in the same genotype.

Table 2. Cotton screening for leaf HC of arid land accessions at USDA, Lubbock, TX.

Lab # , Plot No.	USDA identifier	g avg leaf DW	% yield HC*	g HC yield/ g leaf DW
14954 L1, 16TXLWSA002	DP1212	0.579	4.87	0.028
14955 L2 , 16TXLWSA012	SA-0464	0.542	5.30	0.029
14956 L3 , 16TXLWSA015	SA-0476	0.733	4.54	0.033
14957 L4 , 16TXLWSA016	SA-1049	0.600	3.83	0.023
14958 L5 , 16TXLWSA021	SA-1598	0.530	4.83	0.026
14959 L6 , 16TXLWSA029	STV5458	0.570	4.35	0.025
14960 L7 , 16TXLWSA036	SA-0473	0.493	3.69	0.018
14961 L8 , 16TXLWSA039	SA-1484	0.737	5.78 Hi 1	0.043 Hi
14962 L9 , 16TXLWSA041	SA-1269	0.627	4.08	0.027
14963 L10 , 16TXLWSA042	SA-1555	0.635	3.55	0.023
14964 L11 , 16TXLWSA043	SA-3128	0.632	5.54 Hi 2	0.035
14965 L12 , 16TXLWSA047	SA-2289	0.852	5.06	0.043 Hi
14966 L13 , 16TXLWSA049	FM2011	0.781	3.31 Lo	0.026
14967 L14 , 16TXLWSA050	PHY72	0.627	4.20	0.026
14968 L15 , 16TXLWSA052	SA-1762	0.836	3.49	0.029
14969 L16 , 16TXLWSA053	SA-1759	0.647	3.73	0.024
14970 L17 , 16TXLWSA055	SA-0429	0.682	3.88	0.026
14971 L18 , 16TXLWSA056	STV474	0.471	3.55	0.017 Lo
14972 L19 , 16TXLWSA057	PHY375	0.917	2.88 Lo	0.026
14973 L20 , 16TXLWSA059	SA-2169	0.773	4.59	0.035
14974 L21 , 16TXLWSA062	SA-1599	0.893	4.34	0.039
14975 L22 , Pima,	SJ-FR05	1.018	2.78	0.028
r (leaf wt, % yield) = -0.103 ns				

The survey of USDA germplasm cotton accessions grown with supplemental irrigation at College Station, TX, found % HC yields were very high, with four accessions yielding 11.34, 12.32, 13.23 and 13.73% (Table 3). These HC yields are in the top 2% reported by Adams et al. (1986) and top 1% for sunflowers (Fig. 1, Adams et al. 2017).

Per plant HC yields (g HC/ g leaf DW) varied from 0.023 g to 0.172 g, a 7-fold range (Table 3). Hopi (14992) had a high % HC yield (10.03%), but it was the lowest per plant HC yield (0.023 g/ plant). In contrast, China 86-1 (14997) with the second highest % HC yield (13.23%), had the highest per plant HC yield (0.172, Table 3). The correlation between % HC yield and avg. leaf DW was non-significant (*r* = 0.092 ns). Thus, as seen in the arid land accessions, it appears that one might breed (up to some maximum point) for both % HC yield and leaf DW in cotton. This seems counter intuitive, but it may be that cotton, being a perennial, and closely related to wild plants, may use the leaf hydrocarbons for plant defensive chemicals. If so, there may be an evolutionary advantage to fully protect plants with large leaves as well as those with small leaves. At this survey stage, we have not examined the amount of gossypol (a known defense chemical).

Table 3. Cotton screening for leaf HC at USDA germplasm center, College Station, TX.
For % yield HC: + = 10.01 - 11.00%; ++ = 11.01 - 13.73%.
For g HC yield/leaf DW: + = 0.110 - 0.137g (top 13%); ++ = 0138 - 0.172g (top 3%).

Lab acc Source	USDA identifier	g avg leaf DW (# plants)	% yield HC	g HC yield/ g leaf DW
14983, U1, Tanguisw LMW 12-40	GB-0085	1.335 (4)	5.97	0.080
14984, U2, Mono 57	GB-0204	1.360 (4)	7.37	0.100
14985, U3, Nevis 81	GB-0227	0.728 (4)	10.36 +	0.041
14986, U4, Ashmouni Giza 32	GB-0230	1.128 (4)	7.37	0.083
14987, U5, Ashabad 1615	GB-0790	0.866 (4)	7.01	0.061
14988, U6, Tadla 2	GB-1439	1.106 (4)	9.70	0.107
14989, U7, 3-79	na	0.720 (4)	7.06	0.051
14990, U8, Pima S-5	SA-1497	0.995 (4)	7.92	0.079
14991, U9, TAM 87N-5	SA-1710	0.764 (4)	6.64	0.051
14992, U10, Hopi	SA-0033	0.266 (4)	10.03 +	0.023 Low
14993, U11, Mexican #68	SA-0815	0.994 (4)	7.92	0.079
14994, U12, Christidis 53D7	SA-1166	0.706 (4)	13.73 ++Hi	0.097
14995, U13, Acala SJ-1	SA-1181	0.962 (4)	12.32 ++	0.119 +
14996, U14, 3010	SA-1403	1.463 (4)	9.08	0.133 +
14997, U15, China 86-1	SA-1419	1.300 (4)	13.23 ++	0.172 ++Hi
14998, U16, TM 1	SA-2269	1.244 (4)	11.09 ++	0.138 +
14999, U17, KL 85/335	SA-2589	0.812 (4)	10.25 +	0.083
15000, U18, KLM-2026	SA-2597	0.802 (4)	9.02	0.072
15001, U19,TAM 91C-34	SA-2910	1.006 (4)	10.85 +	0.109
15002,U20,Vir-7080Col.Macias17	SA-3348	0.896 (4)	11.34 ++	0.102
15003, U21,Palmeri, wild	TX-0005	0.398 (5)	7.92	0.032
15004, U22,Latifolium, wild	TX-0100	0.894 (5)	10.72 +	0.096
15005, U23,Latifolium, wild	TX-0104	0.967 (5)	9.25	0.089
15006, U24,Punctatum, wild	TX-0114	0.815 (5)	6.33	0.052
15007, U25,Morrili, wild	TX-0130	0.830 (5)	8.67	0.072
15008, U26,Marie-galante, wild	TX-0367	1.289 (5)	7.37	0.095
15009, U27,Richmondi, wild	TX-0462	0.973 (5)	9.93	0.097
15010, U28,Marie-galante, wild	TX-0866	0.511 (5)	8.05	0.041
15011, U29,Marie-galante, wild	TX-0878	0.692 (5)	4.50	0.031
15012, U30,Yucantanense, wild	TX-1046	0.728 (5)	3.29 Low	0.024 Low
r (leaf wt, % yield) = 0.092 ns				

Principal Coordinate Analysis (PCoA), utilizing 597 SSR bands, of the 30 accessions revealed the accessions are divided into *G. barbadense* and *G. hirsutum* (Fig. 3, left and right) (see Hinze et al., 2016 for further details on molecular marker analysis). The *G. barbadense* samples (8) are all improved accessions. The samples of *G. hirsutum* contain both wild and improved accessions forming a very loose group, but the wild accessions are mostly found in the upper-right quadrant of the ordination (Fig. 3).

Utilizing the g HC/ g leaf DW data, the above average HC yielding accessions are clearly clustered in a tightly grouped set of improved accessions (Fig. 3, dashed oval). Plotting the high and highest yielding samples revealed that all three of the high yielding samples (SA-1181, SA-1403, SA-2269, top 13%) and the highest yielding individual (SA-1419, top 3%) are found in that group (Fig. 3, dashed oval). The discovery of the highest yielding individuals in a group of improved accessions is surprising, in view of the selection for increased cotton seed and fiber yields.

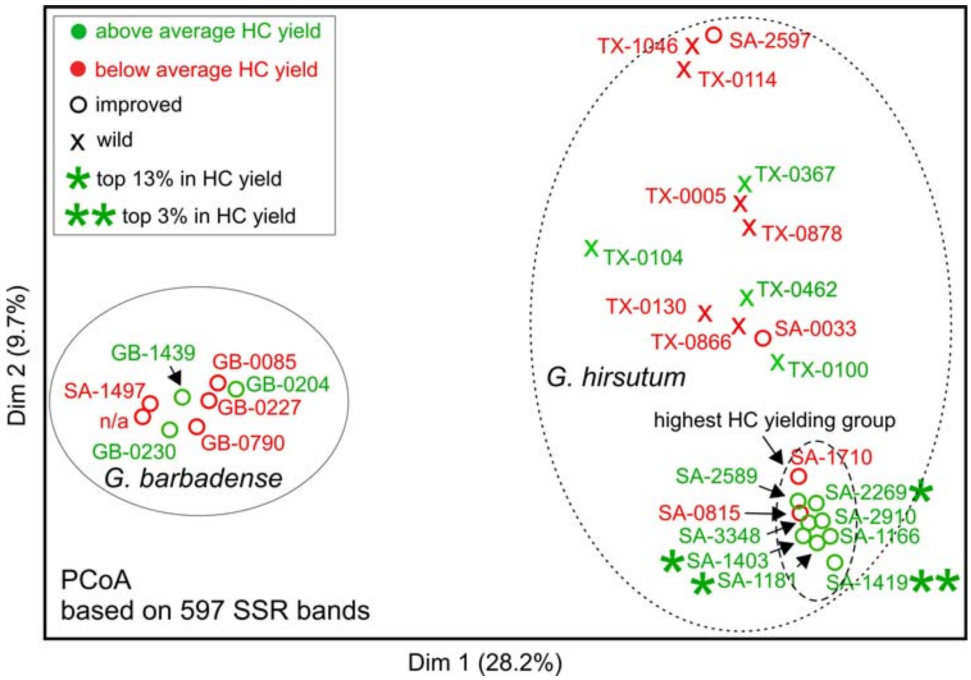

Figure 3. Principal Coordinate Analysis (PCoA) based on 597 SSR bands. The percent of variance accounted for among accessions is given on Dim 1 and Dim 2. See text for discussion.

It is also surprising that none of the wild accessions had high yields, although TX-0100 had a high % yield (10.72%), but having smaller leaves resulted in a moderate total g HC/ g leaf DW yield (Table 3). It is interesting that genetically (by SSR data), TX-0100 is ordinated nearest of any other wild accessions to the high HC yielding group (Fig. 3). It may be that back-crossing TX-0100 with SA-1419 might produce some useful progeny in the future.

CONCLUSION

By the very definition of 'survey', this report is preliminary. Nevertheless, it seems remarkable that a commercial crop, that has been bred and selected for seed (and lint) production, would sequester such high amounts of hydrocarbons in leaves, as found in many cotton accessions. These results raise many evolutionary questions, as well as numerous practical questions such as: Are the HC yields heritable? Are they environmentally induced? Can breeding increase these HC levels without detrimental effects on growth and hardiness? Clearly, much more research is needed (in progress).

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