Comparison of leaf hydrocarbon yields from commercial cotton, FiberMax 1320 treated with growth regulator (Stance®) vs. non-treated plants.

Robert P. Adams and **Amy K. TeBeest**
Baylor University, Biology Department, Baylor-Utah Lab, 2240 E. Cobalt Dr., Suite 25,
St. George, UT 84790
robert_adams@baylor.edu

ABSTRACT

The yields of hexane extractable hydrocarbons from ten plants of commercial cotton, FiberMax 1320, grown dryland in Hansford County, TX and sprayed with growth regulator (Stance®) were compared with ten plants non-treated. Significant differences were not found in leaf biomass or HC yield (as g/ weight 10 DW leaves). However, there was a significant difference (p= 0.05) in % HC yields. Plants **sprayed** with the growth regulator had a **lower** % HC yield. In contrast, plants **not sprayed** had a **higher** % HC yield. The use of a foliar spray containing both mepiquat chloride and cyclanilide (Stance®) resulted in the production decreased amounts of stored HC in cotton. It appears that Stance® not only disrupts gibberellic acid synthesis and the transport of auxins, but likely influences other synthesis pathways (including those leading to stored hydrocarbons). Published on-line www.phytologia.org *Phytologia 100(1): 1-5 (Mar 16, 2018).* ISSN 030319430.

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 derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed how 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.

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.

Adams et al. (2017a) reported 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. They 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.

Adams et al. (2017b) 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.

Adams et al. (2017b) also reported on four accession of cotton (SA-1181, 1403, 1419, and 2269) that 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.

Cotton is a subtropical, perennial, woody plant and as such, has an indeterminate growth pattern. However, in most of the world it is grown as a short life cycle "annual". Excessive vegetative growth can be detrimental as fruit may abort, crop maturity can be delayed leading to a reduced harvest (Jost, et al. 2006).

Plant Growth Regulators (PGRs or GRs) are very widely used in the cultivation of cotton (Rademacher 2015; Jost et al. 2006; Dodds, et al. 2010). PGRs are applied to balance vegetative and reproductive growth. PGRs are used to control excessive vegetative growth (PIX, mepiquat chloride; mepiquat pentaborate; cyclanilide, Stance® (cyclanilide+mepiquat chloride), etc., Rademacher 2015). PIX works by inhibiting cyclases (Rademacher 2015) involved in the synthesis of gibberellic acid (GA), thence leading to loss (decrease) of GA based compounds (eg. GA3, GA4, etc.). GAs promote longitudinal growth in plants (among other factors) (Rademacher 2015). Cyclanilide inhibits transport of natural auxins, and thus, reduces growth in cotton (Rademacher 2015).

In addition, PGRs (eg. Finish®, FreeFall®, Ginstar®, etc.) are used to defoliate cotton to aid harvesting and boll opening.

As an effort to understand the induction of defense chemicals in cotton, we present the results of a field comparison of the effect of spraying a growth regulator (Stance®) on commercial cotton, FiberMax 1320.

MATERIALS AND METHODS

Plant Materials: 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". Ten plants were randomly selected, tagged and a plastic bag was placed over the plants to shield them from an application of growth regulator (Stance®, Bayer CropScience, Inc., Cyclanilide [1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid, 2.1%], Mepiquat chloride [N,N-dimethylpiperidinium chloride, 8.4%], 2 oz. / acre, July 1, 2017. The bags were removed ca. 5 hr after the field was sprayed. Two weeks later (July 16, 2017), ten (10) mature leaves were collected from each of the 10 protected plants and 10 leaves from 10, random plants in the sprayed field. All plants had flowers and a few young bolls. 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). Three grams 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 decanted through a Whatman paper filter into a pre-weighed aluminum pan, and the hexane was evaporated on a hot plate (50°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 (ODW) of 1.085. For the cultivated cotton from Oslo, TX, the shaker yields were corrected by the increased soxhlet extraction efficiency (SEE = x 1.14), for a Correction Factor CF = 1.24 (ODW 1.085 x SEE 1.14 = 1.24). Statistical analyses (means, variance, standard deviation, standard error of mean) were performed by use of EasyCalculation (https://www.easycalculation.com/statistics/standard-deviation.php) and t-tests were performed at socscistatistics.com

RESULTS

Biomass and hydrocarbon (HC) yields for plants sprayed with Growth Regulator (GR) and not sprayed are given in Table 1. No significant difference was found for leaf biomass nor for HC yield (as g/ weight 10 DW leaves. However, there was a significant difference (p= 0.05) for % HC yields. Plants **sprayed** with the growth regulator had a **lower** % HC yield. In contrast, plants **not sprayed** had a **higher** % HC yield.

Because Stance® contains both mepiquat chloride and cyclanilide, each of which acts on different biosynthesis mechanisms (Rademacher 2015), it is probablematical to speculate too much. However, mepiquat chloride is known to inhibit cyclases (Rademacher 2015). It seems likely that other synthesis pathways are disrupted (including those leading to stored, hydrocarbons). If so, this could explain the lower amount of HC in the plants treated with GR (Table 1). Likewise, because cyclanilide inhibits the transport of auxins (Rademacher 2015), it may also influence the pathway(s) leading to the synthesis of HC.

Table 1. Comparison of leaf biomass and HC yields for greenhouse versus field grown cotton.
GR = Growth Regulator (sprayed on plant). ns = non-significant (at p = 0.05), * = significant (at p= 0.05).

	DW 10 lvs/ plant		% HC yield		HC g/ 10 DW lvs	
Material	with GR	no GR	with GR	no GR	with GR	no GR
FiberMax 1320	5.34 g 0.261	5.38 g 0.484	**3.724%** 0.133	**4.277%**, 0.264	0.200 g 0.0115	0.234 g 0.0284
	t-test: p = 0.89 **ns**		t-test: **p=0.025** *		t-test: p=0.106 **ns**	

However, because Stance® suppressed new vegetative growth and favors shorter internodes with few total number of main stem nodes (Jost et al. 2006), the application of Stance® may have merely slightly modified the life cycle of cotton increasing its maturation, and, perhaps signaling the plant to develop the bolls. This shift in life cycle may have led to increased conversion of HC resources in the leaves for use in the developing bolls (Adams et al. 2017b).

These results are illustrated in Figure 1, where one can see the small (non-significant) effects on leaf biomass (g DW 10 lvs) and a significant effect on % HC yield, with the sprayed plants, having a lower amount. The g HC/ DW 10 leaves values are not significantly different, but appear larger in the not sprayed plants (Fig. 1).

Figure 1. Bar graphs of gDW 10 lvs, % HC yield, and HC g/ 10 DW lvs for sprayed vs. not sprayed plants.
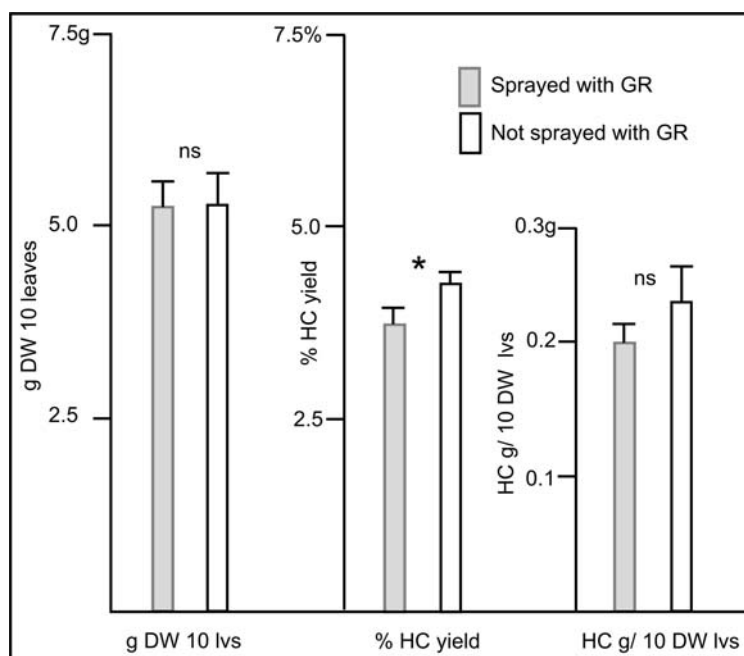
This is the first in a series of studies by our lab to determine the effects of various growth regulators (including herbicides to stress cotton plants). These results, at first, appear counter-intuitive, but upon reflection, the application of Stance®, that contains both mepiquat chloride and cyclanilide, certainly compounded the possible responses within the cotton plants. It is clear that the use of a foliar spray containing both mepiquat chloride and cyclanilide (Stance®) is not useful in promoting increased amounts of stored HC in cotton. Additional research involving a complete examination of the biomass in stems, leaves, flowers, and bolls is needed to better understand the impact of Stance® on cotton metabolism.

ACKNOWLEDGEMENTS

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.

Dodds, D. M., J. C. Banks, L. T. Barber, R. K. Boman, S. M. Brown, K. L. Edmisten, J. C. Faircloth, M. A. Jones, R. G. Lemon, C. L. Main, C. D. Monks, E. R. Norton, A. M. Stewart and R. L. Nichols. 2010. Agronomy and Soils: Beltwide evaluation of commercially available plant growth regulators. J. Cotton Sci. 14: 119-130.

Jost, P., J. Whitaker, S. M. Brown and C. Bednarz. 2006. Use of plant growth regulators as a management tool in cotton. Coop. Ext., U. Georgia, College of Ag., Bulletin 1305.

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.

Rademacher, W. 2015. Plant Growth Regulators: Backgrounds and uses in plant production. J. Plant Growth Regul. 845-872.

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.