The effects of growth regulators on the growth and yields of hydrocarbons in *Cotton* (*Gossypium hirsutum*)

Robert P. Adams and Sam T. Johnson
Baylor-Utah Lab, Baylor University, 201 N 5500 W, Hurricane, UT, 84737, USA
robert_Adams@baylor.edu

ABSTRACT

Glyphosate, 2,4-D, methyl jasmonate, and ethephon, sprayed on leaves of cotton (SA-2269), produced non-significant changes in leaf biomass. These treatments also resulted in lower % HC yields in the order of: control (not sprayed), glyphosate, 2,4-D, methyl jasmonate, 100 ppm Florel and 1000 ppm ethephon. No significant differences were found in g HC/ g DW 10 leaves. Published on-line www.phytologia.org *Phytologia 100(4): 195-198 (Dec 21, 2018).* ISSN 030319430.

KEY WORDS: Cotton, *Gossypium hirsutum*, yields of hexane extractable leaf hydrocarbons, growth regulators, 2,4-D, glyphosate, methyl jasmonate, ethephon.

Cotton (*Gossypium* sp.) 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, and crop maturity can be delayed leading to a reduced harvest (Jost, et al. 2006).

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). 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.

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).

Recently, Adams and TeBeest (2018) reported on the effects of a growth regulator (Stance®) on biomass, % HC yields and g HC/ g leaf biomass in commercial cotton. They reported 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).

This study examined the effects of foliar applications of glyphosate (ex RM43®, 43.68% glyphosate), 2,4-D (Fertilome Weed-Out®, (6.42%, 2,4-D, dimethylamine salt; 2.13% Qinclorac; 0.6% Dicamba, dimethyl amine salt), methyl jasmonate, 100 ppm and 1000 ppm ethephon (Florel® , 3.9% ethephon) on growth and HC yields in cotton (accession SA-2269).

MATERIALS AND METHODS

Plant Materials:

Cotton, SA-2269, was grown in Hurricane, UT, at Connie Stratton's garden plot (37° 09' 34.59" N, 113° 19' 36.63" W, elev. 3265 ft., sandy-loam, soil, watered at needed). An individual plant was bagged with large plastic bags (to prevent spray from drifting onto other plants) hand sprayed until spray wetted and coated each leaf. Five plants in each treatment were sprayed with:

1. 1000 ppm, glyphosate [ex RM43®, 43.68% glyphosate (cf. Roundup®)]. Ragan and Massey, Inc., Ponchatoula, LA
2. 100 ppm, 2,4-D (ex Fertilome Weed-Out®, 6.42%, 2,4-D, dimethylamine salt; 2.13% Qinclorac; 0.6% Dicamba, dimethyl amine salt), VGP, Bonham, TX
3. 100 μM, methyl jasmonate, Sigma-Aldrich, St. Louis, MO., USA
4. 100 ppm, ethephon (ex Florel® , 3.9% ethephon [2-chloroethyl phosphoric acid]), Lawn and Garden Products, Fresno, CA.
5. 1000 ppm, ethephon (ex Florel® , 3.9% ethephon [2-chloroethyl phosphoric acid]), Lawn and Garden Products, Fresno, CA.

The ten (10) lowest growing, non-yellowed leaves were collected and 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 gram aliquot of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane, the jar 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 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. The soxhlet correction factor (sCF) was determined to be 1.14. All shaker extraction yields were corrected to oven dry weight (ODW) by multiplication of 1.085. Thus, the total CF was 1.24 (1.14 x 1.08). ANOVA and SNK (Student-Newman-Keuls multi-range tests) were performed in program SNK (by RPA) as formulated in Steel and Torrie (1960).

RESULTS

Biomass and hydrocarbon (HC) yields for control (not sprayed) and plants sprayed with various Growth Regulators (GR) are given in Table 1. A significantly (P=0.05) larger g DW/ 10 leaves was found in plants sprayed with 1000 ppm ethephon. Percent (%) HC yields, was negatively effected by all GRs. Yields were highest in the control plants, then decline to the lowest yields in 1000 ppm ethephon. Yields based on g HC/ g DW 10 leaves had no significant differences (Table 1).

Table 1. Comparison of leaf biomass and HC yields for cotton (SA-2269) sampled 4 days after spraying with a growth regulator. Any data values that share the same superscript are not significantly differ at P=0.05 by SNK multi-range tests. F (from ANOVA) significance: **=0.01. ns = non-significant (at P = 0.05)

SA-2269, treatment	g DW/ 10 leaves	% HC yield	g HC/ g DW 10 leaves
Control	$3.78g^{B}$	$4.77\%^{A}$	$0.152g^{A}$
1000 ppm glyphosate	$5.45g^{AB}$	$4.24\%^{AB}$	$0.234g^{A}$
100 ppm 2,4-D	$4.23g^{AB}$	$4.19\%^{AB}$	$0.174g^{A}$
100 µM methyl jasmonate	$5.16g^{AB}$	$3.72\%^{B}$	$0.191g^{A}$
100 ppm ethephon	$4.28g^{AB}$	$3.42\%^{B}$	$0.171g^{A}$
1000 ppm ethephon	$5.56g^{A}$	$3.40\%^{B}$	$0.191g^{A}$
F, Significance.	F=2.84 P=0.035 ns	F=4.04 P=0.008**	F= 1.21 P=0.334 ns

This study found that spraying cotton (SA-2269) with glyphosate, 2,4-D, methyl jasmonate, and ethephon resulted in non-significant changes in leaf biomass (although, all treatments resulted in a slightly higher leaf biomass, than in the control). These treatments resulted in lower % HC yields and no significant differences in g HC/ g DW 10 leaves.

ACKNOWLEDGEMENTS

Thanks to Connie Stratton, Hurricane, UT, for assistance in growing the plants in her garden plot. This research funded by Baylor University.

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: 200-207.

Adams, R. P. and A. K. TeBeest. 2018. Comparison of leaf hydrocarbon yields from commercial cotton, FiberMax 1320 treated with growth regulator (Stance®) vs. non-treated plants. Phytologia 100: 1-5.

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.

Majdi, M., M. R. Abdollahi and A. Maroufi. 2015. Parthenolide accumulation and expression of genes related to parthenolide biosynthesis affected by exogenous application of methyl jasmonate and salicylic acid in *Tanacetum parthenium*. Plant Cell. Rep. DOI 10.1007/s00299-015-1837-2.

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.

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.

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