The effects of methyl jasmonate on the growth and yields of hydrocarbons in *Helianthus annuus* (Asteraceae, Sunflowers)

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

Sunflowers, *H. annuus* cv. Little Becka and cv. Munchkin, were sprayed with 100 μM methyl jasmonate (MeJA) to examine the effects MeJA on the free, stored hydrocarbons concentrations. For Little Becka, the % HC yields declined from the control in 2d, further in 4d. Biomass, as estimated by dry weight of 10 leaves/ plant, increased in day 2 and again in day 4 samples. HC yields, as g HC/ g DW 10 leaves, did not differ between control and day 2, but then had a significant drop in day 4. Punching 3 holes per leaf did not effect % HC or g HC, but did result in a significant decline in leaf biomass. For Munchkin, the application of MeJA had no effects on leaf biomass for up to 7 days, then a significant increase after 14 days. It seems likely that this delayed growth was due to normal growth, after the MeJA had dissipated. The % HC yields declined after just 2 days and remained low through day 7, then increased to, statistically, the same level as the control. A similar pattern was seen in g HC/ g DW 10 leaves. MeJA had significant effects on both Little Becka and Munchkin, decreasing the free HC by over 50% in 2 to 4 days, seemingly implying that the application of this growth regulator is not useful in increasing the free HC for alternative fuels from sunflowers. Published on-line www.phytologia.org *Phytologia 100(3):177-182 (Sep 21, 2018).* ISSN 030319430.

KEY WORDS: *Helianthus annuus*, Sunflower, methyl jasmonate, effects on hydrocarbon yields.

In a seminal paper on the induction of sesquiterpene lactone (STL) defenses in *Helianthus annuus*, by surface application of methyl jasmonate (MeJA), Rowe, Ro and Rieseberg (2012) found that MeJA treated sunflower plants had a lower STL production and lower glandular trichome density. This is in contrast to other studies that have found MeJA to induce increased concentrations of terpenoids in cotton (*Gossypium hirsutum*, Opitz, Kunert and Gershenzon, 2008), *Tanacetum parthenium* (Majdi et al. 2015) and see review on the roles of MeJA in plants by Browse (2005).

It appears that defense chemicals are both constitutive and inducible defenses (see Wittstock and Gershenzon, 2002 for discussion). Recently, we reported (Adams et al. 2017c) that progeny of high hydrocarbon (HC) yielding sunflower (*H. annuus*) populations displayed much reduced HC yields when grown in greenhouse conditions. Notice (Fig. 1) that the percent HC (greenhouse / field grown HC yields) decreased to 45.9, 55.6 and 78.3%. In addition, g HC / g DW leaves was very reduced to 6.1 to 17.9% in greenhouse grown plants. It appears that biotic and abiotic factors in natural populations can have large effects on HC yields. With this in mind, it seemed of interest to investigate the effects of MeJA on HC yields from greenhouse grown sunflowers.

This is a part of a continuing study on the development of sunflowers as a source for natural rubber and bio-fuels from the biomass (Adams et al., 1986; Adams and Seiler, 1984; Adams and TeBeest, 2016; Adams et al. 2016; Adams and TeBeest, 2017; Adams et al. 2017a,b,c; Adams et al. 2018a,b,c; Pearson et al., 2010a,b; Seiler, Carr and Bagby, 1991,).

MATERIALS AND METHODS

Seeds of *H. annuus* cv. Little Becka and cv. Munchkin were obtained from Burpee Seed Co., Warminster, PA and Sunflower Selections, Inc., Woodland, CA, respectively. Seeds were planted in 6 " square plastic pots using Miracle Grow® potting soil. Plants were grown in a growth chamber with LED lighting approximately equal to daylight for 16 hr light, 8 hr dark cycles and watered as needed.

The lowest growing, non-yellowed, 10 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays when the HC yields are reported as highest for sunflowers (Adams et al. 2016). The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Rowe, Ro and Rieseberg (2012) used 100 μM methyl jasmonate (Sigma-Aldrich, Ontario, Can), sprayed on leaves until wet. Their procedure was followed in this study. Adams et al. (2016) have shown that the maximum yields of HC occurs at first flower (R5.1), so all sampling was done at first flower for Little Becka and Munchkin plants.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were 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 filtered 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 pre-weighed aluminum pan with concentrated hydrocarbon extract was weighed and tared. Extraction of identical samples by shaking and soxhlet (8 hr) yielded a correction factor of 1.9 (soxhlet yield/ shaking yield), which when corrected to oven dry weight basis (ODW) by 1.085 resulted in a total correction factor of 2.06.

ANOVA and SNK (Student Newman-Keuls) multiple range tests were programmed following the formulations in Steel and Torrie (1960).

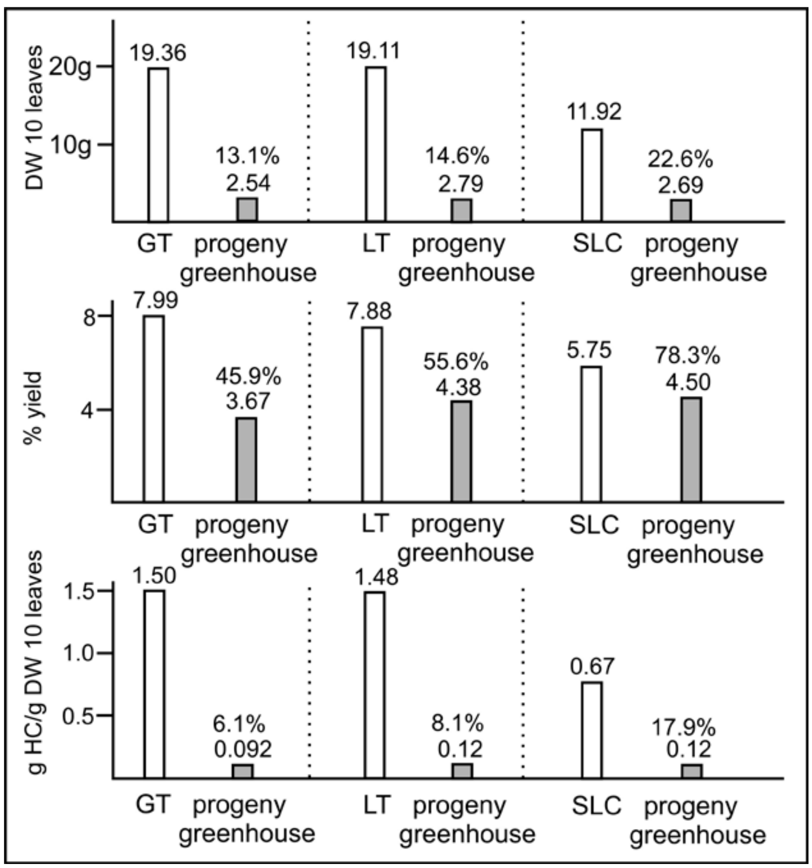

Figure 1. Comparison of DW 10 leaves, HC yield and g HC/ gDW 10 leaves for fields sampled sunflowers vs. their progeny grown in the greenhouse at OPSU (adapted from Adams et al. 2017c).

RESULTS

Table 1 presents the effects of MeJA on leaf biomass, % HC yields, and g HC/ g DW 10 leaves for cv. Little Becka. Each of these three variables were very highly significantly effected. Graphic analyses of these effects are shown in Fig. 2. Notice biomass, as estimated by dry weight of 10 leaves/ plant, increased in day 2 and again in day 4 samples (Fig. 2, solid line). The % HC yields declined from the control to 2d, further in 4d (Fig. 2, dotted line). HC yields, as g HC/ g DW 10 leaves, did not differ between control and d2, but did display a significant drop in d4 (Fig. 2, small dotted line). Punching 3 holes per leaf did not effect % HC or g HC, but did result in a significant decline in leaf biomass.

Table 1. Comparison of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Little Becka sprayed with 100 µM MeJA and analyzed after 2 and 4 days, plus plants sprayed and with leaves punctured with a hole punch, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05).

Little Becka component	control, no treatment	+ MeJA coll. 2 days later	+MeJA coll. 4 days later	+MeJA + holes in leaves, coll. 4 days later	F ratio, significance
wt. 10 leaves	1.72g^{b}	2.05g^{b}	2.76g^{a}	2.03g^{b}	F= 5.67 P = 0.47 x 10^{-2}***
% HC yield	3.31%^{d}	2.41%^{e}	0.92%^{f}	1.10%^{f}	F= 25.4 P=0.5 x 10^{-5} ***
g HC/ g 10 leaves	0.055g^{x}	0.051g^{x}	0.025g^{y}	0.022g^{y}	F= 10.6 P=0.25 x 10^{-3} ***

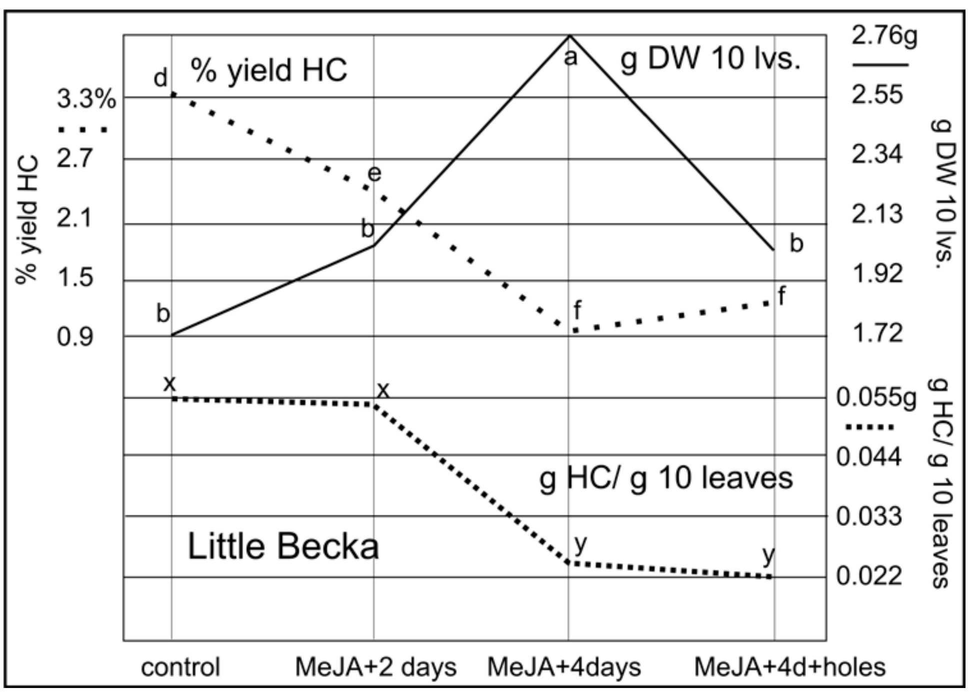

Figure 2. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Little Becka sprayed with 100 µM MeJA and analyzed after 2 and 4 days, plus plants with holes in the leaves and also sprayed with MeJA, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05). See text for discussion.

Table 2 presents the effects of MeJA on leaf biomass, % HC yields, and g HC/ g DW 10 leaves for cv. Munchkin. Each of these three variables were very highly significantly affected. Graphic analyses of these effects are shown in Fig. 3. Notice (Fig. 3) MeJA had no effects on leaf biomass for up to 7d, then a significant increase after 14d. It seems likely that this delayed growth was due to normal growth, after the MeJA had dissipated. The % HC yields declined after just 2d and remained low through day 7, then increased to, statistically, the same level as the control (Fig. 3). A similar pattern was seen (Fig. 3) in g HC/ g DW 10 leaves (Fig. 3).

Table 1. Comparison of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Little Becka sprayed with 100 μM MeJA and analyzed after 2 and 4 days, plus plants sprayed and with leaves punctured with a hole punch, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05).

Munchkin component	control, no treatment	+ MeJA coll. 2 days later	+MeJA coll. 4 days later	+MeJA coll. 7 days later	+MeJA coll. 14 days later	F ratio, significance
wt. 10 leaves	2.63g^{b}	2.36g^{b}	2.53g^{b}	2.46g^{b}	4.06g^{a}	F= 12.4 P = 0.41x10^{-4} ***
% HC yield	4.36%^{d}	3.32%^{ef}	3.26%^{ef}	2.99%^{f}	3.91%^{de}	F= 6.76 P=0.90 x 10^{-3} ***
g HC/ g 10 leaves	0.113g^{y}	0.077g^{z}	0.081g^{z}	0.073g^{z}	0.159g^{x}	F= 28.83 P=0.89 x 10^{-6} ***

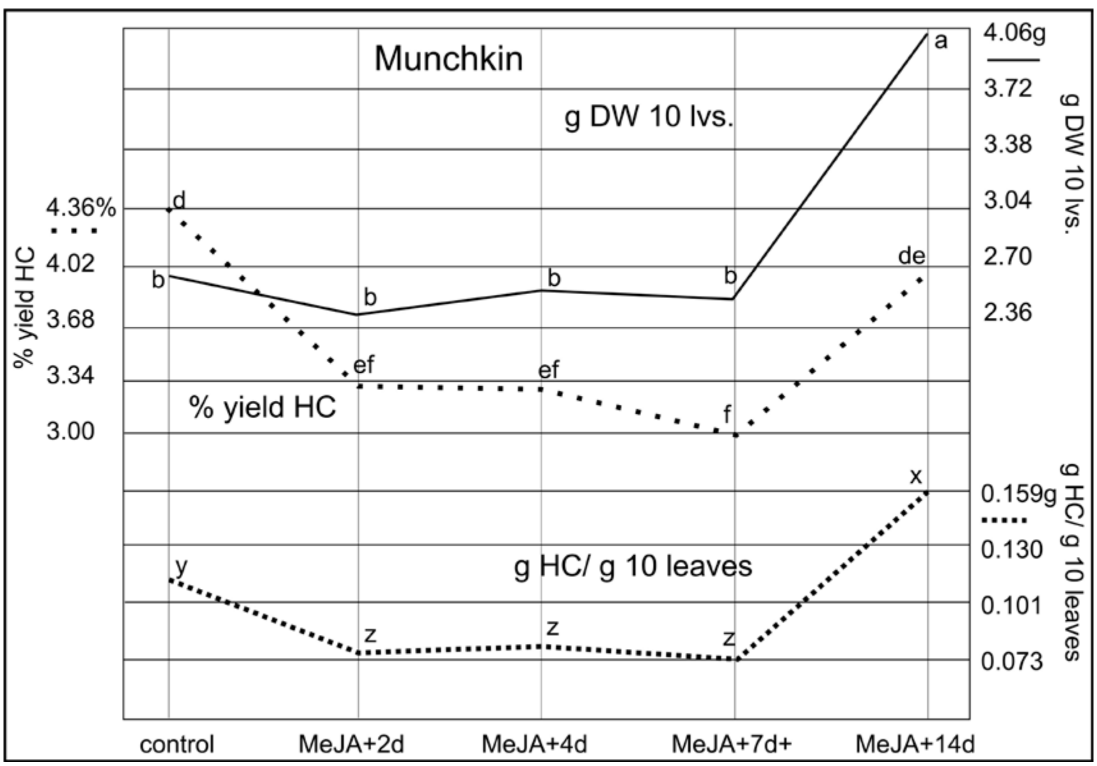

Figure 3. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Munchkin sprayed with 100 μM MeJA and analyzed after 2 and 4 days, plus plants with holes in the leaves and also sprayed with MeJA, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05). See text for discussion.

This research was initiated in the hope of stimulating the production of HC (as secondary compounds). However, although MeJA had significant effects on both Little Becka and Munchkin, unfortunately, it decreased the free HC by over 50% in 2 to 4 days. This seems to imply that the application of MeJA is not useful in increasing the free HC for alternative fuels from sunflowers.

ACKNOWLEDGEMENTS

Thanks to Connie Stratton for care in growing the plants. This research supported by funds from Baylor University (0324512 to RPA).

LITERATURE CITED

Adams, R. P., M. F. Balandrin, K. J. Brown, G. A. Stone and S. M. Gruel. 1986. Extraction of liquid fuels and chemical from terrestrial higher plants. Part I. Yields from a survey of 614 western United States plant taxa. Biomass 9: 255-292.

Adams, R. P. and G. J. Seiler. 1984. Whole plant utilization of sunflowers. Biomass 4:69-80.

Adams, R. P. and A. K. TeBeest. 2016. The effects of gibberellic acid (GA3), Ethrel, seed soaking and pre-treatment storage temperatures on seed germination of *Helianthus annuus* and *H. petiolaris*. Phytologia 98: 213-218.

Adams, R. P., A. K. TeBeest, B. Vaverka and C. Bensch. 2016. Ontogenetic variation in hexane extractable hydrocarbons from *Helianthus annuus.* Phytologia 98: 290-297

Adams, R. P. and A. K. TeBeest. 2017. The effects of different concentrations of gibberellic acid (GA3) on seed germination of *Helianthus annuus* and *H. petiolaris* Phytologia 99: 32-35.

Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet, C. Parker and D. Thornburg. 2017a. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 1-10.

Adams, R. P., A. K. TeBeest, W. Holmes, J. A. Bartel, M. Corbet and D. Thornburg. 2017b. Geographic variation in volatile leaf oils (terpenes) in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99: 130-138.

Adams, R. P., A. K. TeBeest, T. Meyeres and C. Bensch. 2017c. Genetic and environmental influences on the yields of hexane extractable hydrocarbons of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 99(2): 186-190.

Adams, R. P., A. K. TeBeest, S. McNulty, W. H. Holmes, J. A. Bartel, M. Corbet, C. Parker, D. Thornburg and K. Cornish. 2018a. Geographic variation in natural rubber yields in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers). Phytologia 100: 19-27.

Adams, R. P., Matt Lavin and Gerald P. Seiler. 2018b. Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers) II. Phytologia 100(2): 153-160.

Adams, R. P., Matt Lavin, Steve Hart, Max Licher and Walter Holmes. 2018c. Screening hydrocarbon yields of sunflowers: *Helianthus maximiliani* and *H. nuttallii* (Asteraceae). Phytologia 100(2): 161-166.

Browse, J. 2005. Jasmonate: an oxylipin signal with many roles in plants. Plant Hormones 72: 431-456.

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.

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.

Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath and M. Whalen. 2010a. Natural rubber quantification in sunflower using automated solvent extractor. Indust. Crops and Prods. 31: 469-475.

Pearson, C. H., K. Cornish, C. M. McMahan, D. J. Rath, J. L. Brichta and J. E. van Fleet. 2010b. Agronomic and natural rubber characteristics of sunflower as a rubber-producing plant. Indust. Crops and Prods. 31: 481-491.

Rowe, H. C., Ro, D-K and L. H. Rieseberg. 2012.

Seiler, G. J., M. E. Carr and M. O. Bagby. 1991. Renewables resources from wild sunflowers (*Helianthus* spp., Asteraceae). Econ. Bot. 45: 4-15.

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

Whittstock, U. and J. Gershenzon. 2002. Constitutive plant toxin and their role in defense against herbivores and pathogens. Curr. Opin. Plant Biol. 5: 300-307.