# Ontogenetic variation in pentane extractable hydrocarbons from *Helianthus annuus*

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## ABSTRACT

Yields of total hydrocarbons (HC) from leaves of *Helianthus annuus* cv. Little Becka and cv. Firecracker reached their maximum for both % yields and g HC/ plant at first flowering (stage R 5.1). Hydrocarbon yields, as % yields and as g/ plant, showed nearly identical patterns with ontogeny. Yields of HC (g/ plant) from leaves plus seed heads reached a maximum with seed filling at R 6 stage (rays wilted). Biomass of leaves, stems and seed heads were examined and total biomass was found to reach a maximum at stage R 6 (rays wilted), with a slight decline in biomass at stage R 8 (seeds filled, head nodding). The optimum time to sample leaves for hydrocarbons is at first flowering (R 5.1), when leaf hydrocarbons are at a maximum. Published on-line www.phytologia.org *Phytologia 98(4): 290-297 (Oct 6, 2016).* ISSN 030319430.

**KEY WORDS**: *Helianthus annuus,* Sunflower, ontogenetic variation in leaf hydrocarbon storage.

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Due to the uncertain crude oil production in the United States, there is a renewed interest in sustainable, renewable resources fuel and petrochemicals. Sunflowers have been developed as an important crop, primarily for their oil and edible seeds (Heiser et al. 1969). The triglycerides have been used as bio-diesel (Hoffman et al 1980; Morgan and Shultz, 1981).

Adams and Seiler (1984) surveyed 39 taxa of sunflowers for their cyclohexane (hydrocarbon) and methanol (resins) concentrations. The highest cyclohexane (bio-crude) yielding taxa were *H. agrestis*, an annual, Bradenton, FL (7.38%) and *H. annuus*, Winton, OK (7.09%). Adams et al. (1986) screened 614 taxa from the western US for their hydrocarbon (hexane soluble) and resin (methanol soluble) yields. They reported 2 plants of *H. annuus* from Idaho with 8.71% and 9.39% hydrocarbon yields.

Seiler, Carr and Bagby (1991) reported on 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. The rubber was found to be of lower molecular weight than *Hevea* rubber, but still appeared to be useful as a plasticizing additive and for coatings inside pipes and containers.

Pearson et al. (2010a) demonstrated that Accelerated Solvent Extraction (ASE) could be utilized for the quantification of natural rubber in sunflower. Agronomic and rubber characteristics were reported for *H. annuus* by Pearson et al. (2010b). They reported from 0.9% to 1.7% rubber in sunflower cultivars (Fig. 4, Pearson et al. 2010b).

There does not appear to be any information on the production of hydrocarbons during ontogenetic development of *H. annuus*. The purpose of this report is to present new information on ontogenetic variation of the yields of pentane extractable hydrocarbons in four sunflower cultivars. The major focus of this research was to determine a stage of development when yields of hydrocarbons are at or near their maximum so plant collections could be taken at comparable growth stages across geographic regions.

## MATERIALS AND METHODS

Seed was obtained from nursery seed dealers for 4 cultivars: Firecracker, Hopi, Little Becka and Sunrich Orange. Seeds were germinated in 1" tubes, then seedlings were transplanted (at 2" tall) into 6" plastic pots using a commercial potting soil (Berger BM 7). Plants were watered as needed to avoid wilting. No fertilizer or pesticide spray was added. Firecracker and Little Becka were grown in the greenhouse at Oklahoma Panhandle State University (OPSU) under ambient sunlight with 50% shade screen (spring, 2016). Hopi and Sunrich Orange were grown in the Baylor greenhouse at Oslo, TX under ambient light with 50% shade screen. Six plants were randomly selected for analysis at 5 growth stages (see Schnetter and Miller, 1981 for a description of stages): 1. 1st flower bud mature but not opened (R 3, see Fig. 1); 2. 1st flower opened with mature rays (R 5.1); 3. head nearly (90%) filled with disk flowers (R 5.9); 4. rays wilted on 1st flower (R 6); 5: terminal head yellow and nodding (R 8) (seeds filled).

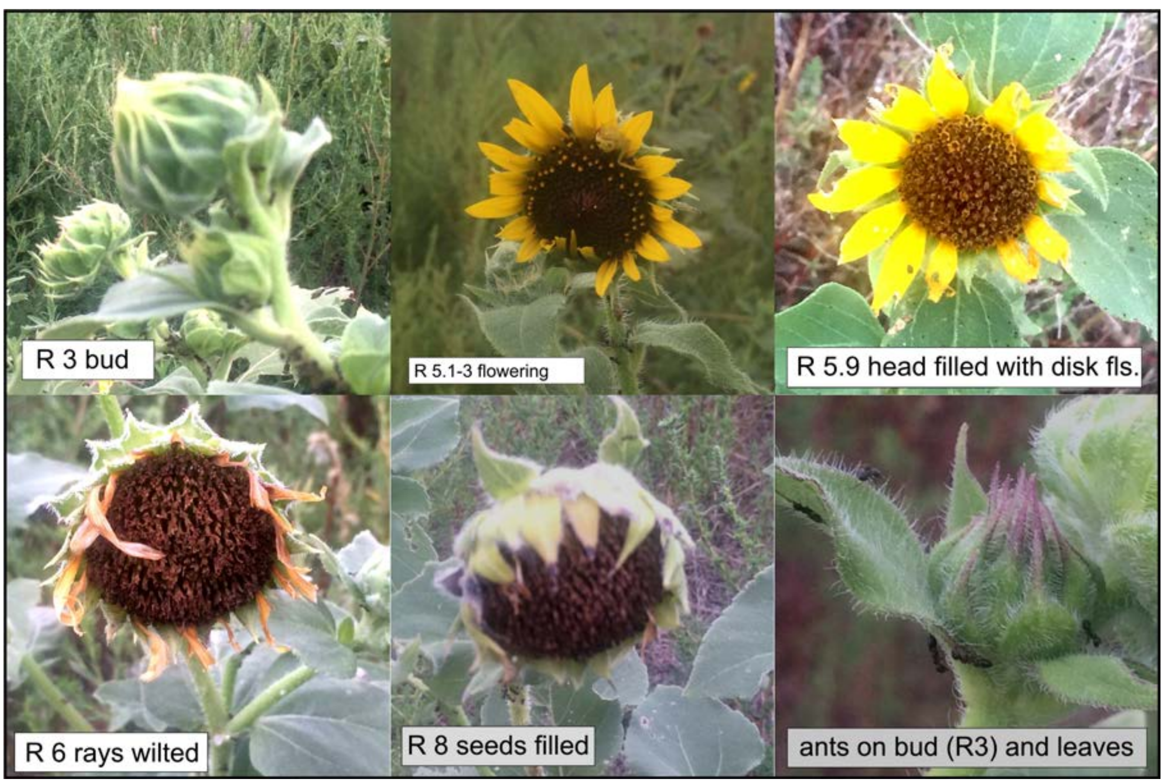


Figure 1. Sampling times at growth stages of wild (*H. annuus*) sunflowers, Gruver, TX. Note black ants on the bud and leaves in lower right photo.

The six plants were divided into leaves, stems, and heads and each part dried (24 h, 45-50° C) then weighed. Leaves and heads 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.

## RESULTS

Tables 1 and 2 contain growth and yields for Little Becka and Firecracker cultivars. Notice (Fig. 2, upper graph) that both cultivars show a maximum % yields of HC from leaves at the first flower stage (R 5.1) with a decline in % yield as the disk flowers develop (R 5.9). The yield increased (Fig. 2) in Little Becka at R 6 (ray flowers wilted), then declined with the filling of seeds (R 8).

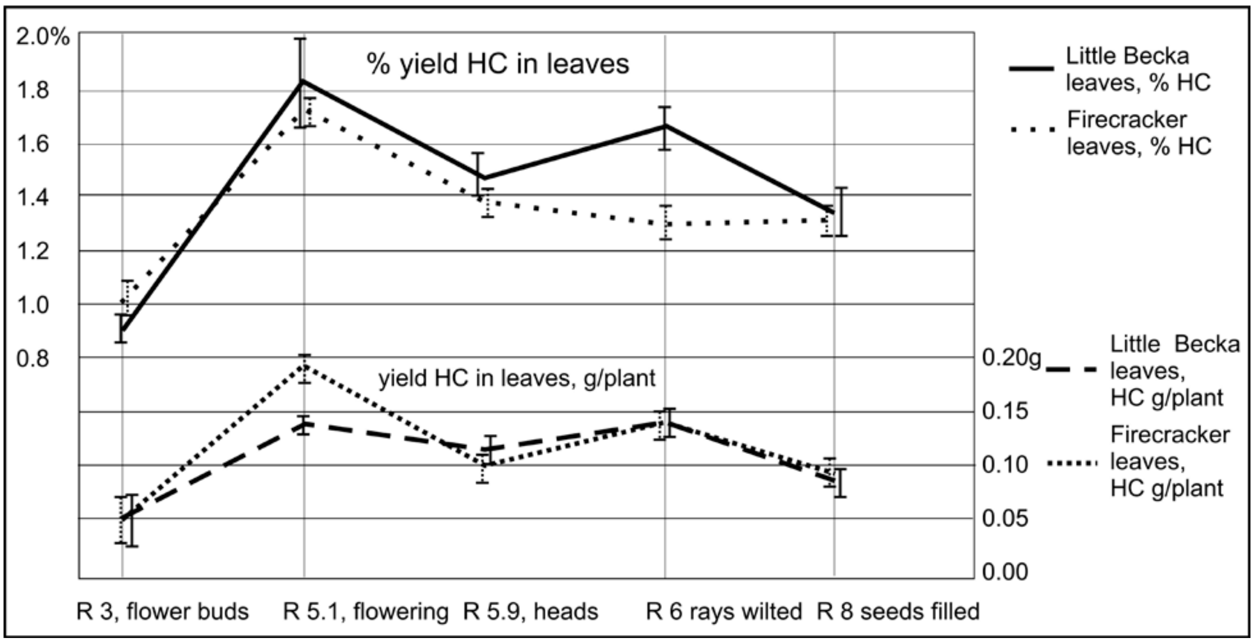


Figure 2. Comparison of hydrocarbon (HC) % yields (upper) between Little Becka and Firecracker cv. Bars are standard error the mean.

The pattern for total HC yields (g/plant) from leaves mirrored the % yield pattern with the g/ plant yields maximized at stage R 5.1 (Fig. 1, Tables 1, 2). A slight, non-significant, rise in HC is suggested at stage R 6 (Fig. 1).

Examination of total g of HC from stems was not a focus of this study. However, we did find that for Firecracker, at stage R 3, the % HC yield from leaves was 1.11% vs. 0.51% from stems. Of course in a farming operation, stems and leaves would be swathed and baled together for processing. As it is far simpler to collect, dry and grind only leaves, that will be the focus in future studies in screening wild sunflower plants.

Yields of HC (g/ plant) from leaves plus seed heads reached a maximum (Fig. 2) with seed filling at R 6 stage (rays wilted). The first sampling of seed heads (R 5.1, first flower opening) is before any seeds are formed. The rather large amounts of HC (Fig. 3, Table 1) of 2.92% in heads vs. 1.81% from leaves seems to be due to large amounts of resin in the flower head bracts. In fact, resin is often excreted and appears to attract small black (sugar) ants (Fig. 1, lower right). The resin is also on the stem and at the base of the leaf blades where black ants congregate. The increase in seed head HC at stage R 6 may be largely due to the synthesis of triglycerides (fats) in the maturing sunflower seeds (stage R 6, Fig. 3, Table 1).

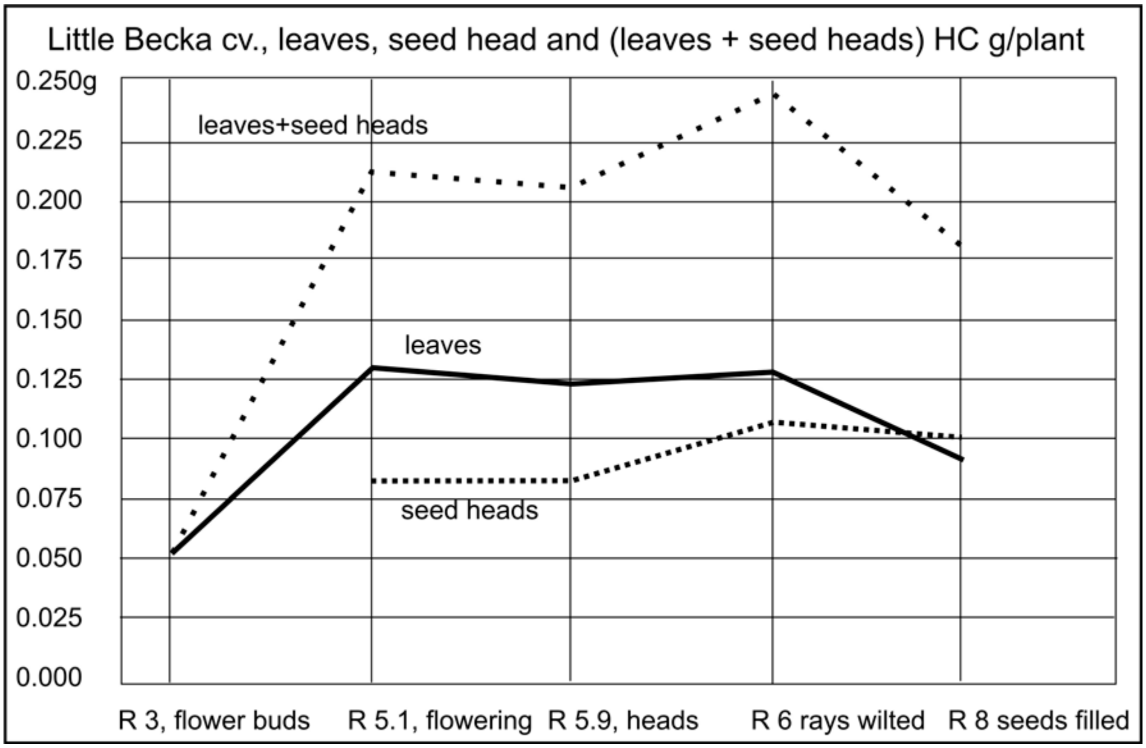


Figure 3. Distribution of HC (g/plant) in leaves, seed heads and leaves + seed heads in Little Becka during the growing season.

The growth and HC yields for Sunrich Orange and Hopi cv. are given in Tables 3, 4. Both of experiments were flawed due to lower ambient light levels that caused the plants to elongated their leaf internodes. This may have affected the levels of HC in the leaves and heads.

Partitioning of biomass between leaves, stems and seed heads for Little Becka and Firecracker is shown in Figs. 4 and 5. Both cultivars show similar patterns, in that leaf biomass declines when flowering commences, stem biomass remains relative stable, and seed head biomass increases as seeds are being filled. For Little Becka, leaf, stem and seed head biomass are about equal at stage 8 (1/3 each, table 1, Fig. 4.). However, for Firecracker at stage 8, seed head biomass is 46.2%, compared to just 20.2% for leaves (Table 2, Fig. 5). It is interesting that the mass (g wt./plant) of stems and leaves both actually decline as the seeds fill (Tables 1, 2). This seems likely due to the transport of sugars and other metabolites from leaves and the stem to the seed heads.

Because Sunrich Orange and Hopi produced elongated stems under sub-optimum light, the biomass of the stem is often greater than the leaf biomass (Tables 3, 4). Notice (Table 3) at stage 8, Sunrich Orange has allocated biomass as follows: leaves, 22.2%; stems, 31.2%; and seed heads, 46.4%. In contrast, Hopi (Table 4) allocated: leaves, 13.3%; stems, 59.8%; and seed heads, 26.9%.

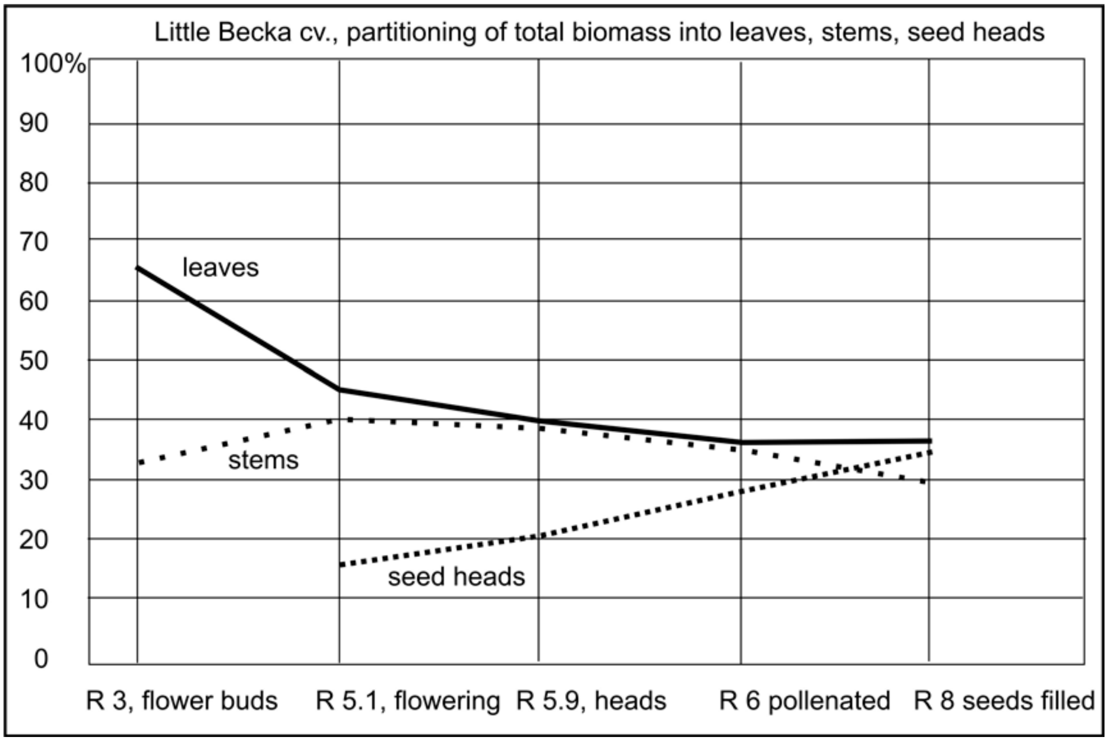


Figure 4. Partitioning biomass among leaves, stems and seed heads during the growing season for Little Becka.

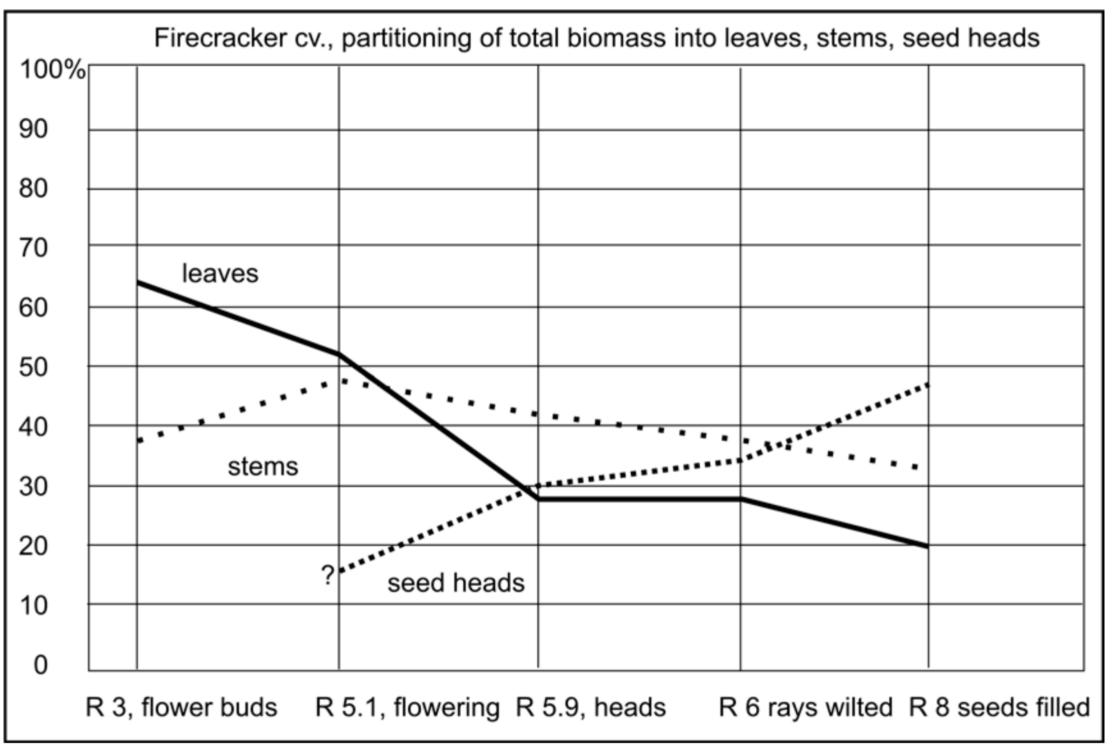


Figure 5. Partitioning biomass among leaves, stems and seed heads during the growing season for Little Becka.

The total biomass varied by stage and among cultivars (Tables 1-4, Fig. 6). Firecracker had the largest changes in biomass, reaching a maximum at R 6, then declining at R 8. This decline in biomass appears mostly due to the decrease in biomass for both leaves and stems. In sunflowers, lower leaves turn yellow as the seeds are filled in the heads. Little Becka and Sunrich Orange show similar patterns to Firecracker by having their peak biomass at R 6, then decline in total biomass at R 8 (Fig. 6). Hopi displayed a slightly different pattern (Fig. 6), increasing from R 6 to R 8 (Fig. 6).

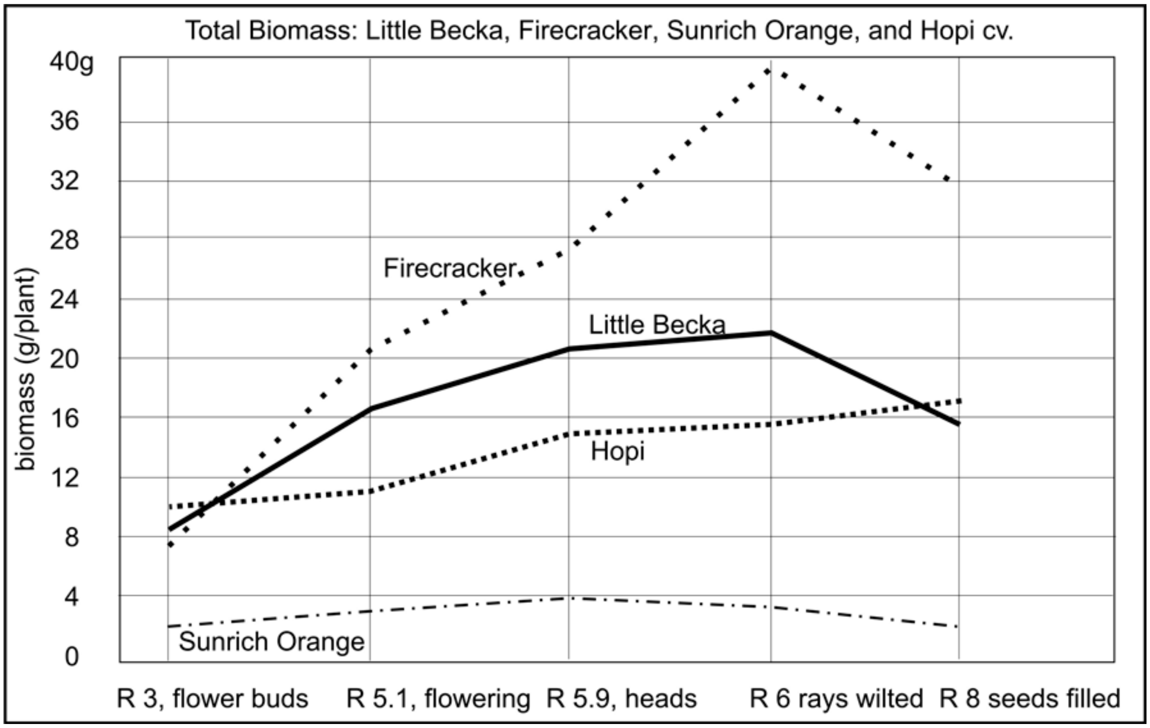


Figure 6. Variation in total biomass with plant maturity and among cultivars.

## CONCLUSION

The primary focus of this study was to determine the optimum time to collect leaves to maximize HC yields. Yields of total hydrocarbons (HC) from leaves of *Helianthus annuus* cv. Little Becka and cv. Firecracker reached their maximum for both % yields and g HC / plant at first flowering (stage R 5.1). Hydrocarbon yields from leaves (as g/ plant) showed a very similar trend. Yields of HC (g/ plant) from leaves plus seed heads reached a maximum seed filling at R 6 stage (rays wilted). Total biomass of leaves, stems and seed heads was examined and biomass was found to reach a maximum at stage R 6 (rays wilted), with a slight decline in biomass at stage R 8 (seeds filled, head nodding). The optimum time to sample leaves is at the first flowering on a plant (R 5.1), when leaf hydrocarbons are at a maximum.

## LITERATURE CITED

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

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.

Heiser, C. B. Jr., D. M. Smith, S. B. Clevenger and W. D. Martin, Jr. 1969. The North American sunflowers (*Helianthus*). Mem. Torrey Bot. Club 22: 1-218.
Hoffman, V., W. D. Dinusson, D. Zimmerman, D. L. Helgeson and C. Fanning. 1980. Sunflower oil as a fuel alternative. Coop. Ext. Ser. Cir. AE-694.
Morgan, R. P. and E. G. Shultz, Jr. 1981. Fuels and chemicals from novel seed oils. Chem. Eng. News 59: 69-77.
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.
Schnetter, A. A. and J. F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21: 901-903.

Table 1. Growth, biomass distribution and HC yields for Little Becka cv. Biomass is based on all leaves or seed heads from each of six plants.

| Little Becka growth stage | biomass leaves wt. (% total) | biomass stem(s) (% total) | biomass heads (% total) | total biomass g/plant | % yield, pentane shaker leaves | % yield pentane shaker flowers/ heads | HC yield ex total leaves/ plant | HC yield ex total heads/ plant | HC yield lvs + heads/ plant |
|---|---|---|---|---|---|---|---|---|---|
| R 3 bud | 5.94g (66.2%) | 3.02g (33.8%) | na | 8.96 | 0.88 | na | 0.052g | na | 0.052g |
| R 5.1-5.3 | 7.46g (43.6%) | 6.90g (40.3%) | 2.76 (16.1%) | 17.12 | 1.81 | 2.92 | 0.135g | 0.081g | 0.216g |
| R 5.9 | 8.66 (40.2%) | 8.43 (39.1%) | 4.46 (20.7%) | 21.55 | 1.43 | 1.89 | 0.123g | 0.084g | 0.207g |
| R 6 | 8.16 (36.9%) | 7.96 (36.1%) | 5.98 (27.01%) | 22.10 | 1.64 | 1.86 | 0.134g | 0.111g | 0.245g |
| R 8 | 5.83 (37.1%) | 4.65 (29.5%) | 5.26 (33.4%) | 15.74 | 1.35 | 1.94 | 0.079g | 0.102g | 0.181g |

Table 2. Growth, biomass distribution and HC yields for Firecracker cv. Based on 6 plants. HC yield per plant from leaves vs. stems was 0.051g vs. 0.014g (3.6:1).

| Fire-cracker growth stage | biomass leaves wt. (% total) | biomass stem(s) (% total) | biomass heads (% total) | total biomass g/plant | % yield, pentane shaker leaves | % yield pentane shaker heads | HC yield ex total leaves/ plant | HC yield ex total heads/ plant | HC yield lvs + heads/ plant |
|---|---|---|---|---|---|---|---|---|---|
| R 3 bud | 4.63g (62.4%) | 2.78g (37.6%) | na | 7.41 | 1.11 | na | 0.051g | na | 0.051g |
| R 5.1-5.3 | 11.05* (52.1%) | 10.18 (47.9%) | na | 21.23 | 1.73* | na | 0.191g | na | 0.191g |
| R 5.9 | 7.49 (27.0%) | 11.86 (42.8%) | 8.38 (30.2%) | 27.73 | 1.38 | 2.01 | 0.103g | 0.168g | 0.271g |
| R 6 | 10.66 (27.2%) | 15.15 (38.6%) | 13.43 (34.2%) | 39.24 | 1.27 | 1.47 | 0.135g | 0.197g | 0.232g |
| R 8 | 6.37 (20.2%) | 10.58 (33.6% | 14.57 (46.2%) | 31.52 | 1.32 | 1.78 | 0.084g | 0.260g | 0.344g |

*with flowering heads included.

Table 3. Growth, biomass distribution and HC yields for Sunrich Orange cv. Based on 6 plants. Plants with elongated stems due to low light in greenhouse.

| Sunrich Orange growth stage | biomass leaves wt. (% total) | biomass stem(s) (% total) | biomass heads (% total) | total biomass g/plant | % yield, pentane shaker leaves | % yield pentane shaker heads | HC yield ex total leaves/ plant | HC yield ex total heads/ plant | HC yield lvs + heads/ plant |
|---|---|---|---|---|---|---|---|---|---|
| R 3 bud | 1.20g (45.1%) | 1.46g (54.9%) | na | 2.66 | 1.77 | na | 0.021g | na | 0.021g |
| R 5.1-5.3 | 1.66* (51.7%) | 1.55 (48.3%) | na | 3.21 | 1.97* | na | 0.033g* | na | 0.033g |
| R 5.9 | 2.51* (60.3%) | 1.65 (39.7%) | na | 4.16 | 1.56* | na | 0.039g* | na | 0.039g |
| R 6 | 0.74 (19.5%) | 1.45 (38.2%) | 1.61 (42.3%) | 3.80 | 1.77 | 1.80 | 0.013g | 0.029g | 0.042g |
| R 8 | 0.59 (22.4%) | 0.82 (31.2%) | 1.22 (46.4%) | 2.63 | 2.01 | 2.14 | 0.012g | 0.026g | 0.038g |

*with flowering heads included.

Table 4. Growth, biomass distribution and HC yields for Hopi cv. Based on 6 plants. Plants with very elongated stems due to low light in greenhouse.

| Hopi growth stage | biomass leaves wt. (% total) | biomass stem(s) (% total) | biomass heads (% total) | total biomass g/plant | % yield, pentane shaker leaves* | % yield pentane shaker heads | HC yield ex total leaves*/ plant | HC yield ex total heads/ plant | HC yield lvs + heads/ plant |
|---|---|---|---|---|---|---|---|---|---|
| R 3 bud | 2.80 (27.7%) | 7.36 (73.3%) | na | 10.16 | 1.73* | na | 0.048g* | na | 0.048g |
| R 5.1-5.3 | 1.91 (17.8%) | 8.14 (75.9%) | 0.68 (6.3%) | 10.73 | 1.67* | na | 0.043g* | na | 0.043g |
| R 5.9 | 2.07 (14.1%) | 11.37 (68.2%) | 1.31 (8.8%) | 14.75 | 1.56* | na | 0.053g* | na | 0.053g |
| R 6 | 2.58 (17.0%) | 10.58 (69.5%) | 2.07 (13.5%) | 15.23 | 1.42* | na | 0.066g* | na | 0.066g |
| R 8 | 2.33 (13.3%) | 10.49 (59.8%) | 4.73 (26.9%) | 17.55 | 3.51* | na | 0.248g* | na | 0.028g |

*with flowering heads included.