VARIATION IN OIL AND OIL QUALITY OF WILD ANNUAL SUNFLOWER (*HELIANTHUS ANNUUS* L.) POPULATIONS IN A UNIFORM ENVIRONMENT.

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ABSTRACT

Thirty-eight populations of wild *Helianthus annuus* L. indigenous to areas from 29°N to 46°N latitude and 81°W to 122°W longitude, were grown at Bushland, Texas, in 1980. Chronological development of oil and oil quality was examined. Flowering heads were sibbed at 50% flower for each population for each week of flowering and seeds were harvested 28 days later. Total oil content averaged over all populations increased from late July through mid-October. Stearic acid (18:0), a polysaturated fatty acid, varied over the season. During the early part of the sampling period (July — mid-August), stearic acid decreased slightly but increased continually thereafter until the last three samplings (Sept. 21 and 28, Oct. 5) when it remained relatively constant. Palmitic acid (16:0), another polysaturated fatty acid, showed much the same trends as stearic acid. The polyunsaturated fatty acids, linoleic (18:2) and oleic (18:1), showed developmental trends different from the saturated acids. Oleic steadily decreased while linoleic increased over the season. Developmental patterns for oil and fatty acids in wild populations of *H. annuus* follow much the same trends that exist in modern commercial hybrid sunflower.

Oil content and oil quality of seeds of the original populations were compared to sibbed and open-pollinated populations of the original population grown in a uniform environment. Oleic acid was the only acid for which mean values from the original population did not differ from mean values of the sibbed and open-pollinated populations. Mean values of linoleic acid in the original population were significantly different from the mean values of linoleic acid of the sibbed and open-pollinated populations. Sibbing would not be necessary for evaluation of oleic and linoleic acids in a uniform environment because their values were not significantly different from open-pollinated populations.

INTRODUCTION

Wild *Helianthus* germplasm, besides constituting the basic stock from which cultivated sunflower originated, continues to contribute specific characteristics for sunflower improvement. Despite the widespread use of wild sunflower species, no significant modifications have been made in the chemical composition of sunflower seed or oil during the development of cultivars and hybrids (Dorrell and Whelan, 1978). Nevertheless, since wild *H. annuus* and other sunflower species appear to possess considerable variability for most economic characteristics, the use of wild material in breeding programs has the potential for markedly changing quality characteristics.

Oil content and oil quality of wild *H. annuus* has been reported by several workers (Pustovoit and Krasnokutskaya, 1976; Dorrell and Whelan, 1978; Knowles *et al.*, 1970; Fick *et al.*, 1976; Thompson *et al.*, 1978; Thompson *et al.*, 1981). Thompson *et al.*, (1978, 1981) reported that seed oil of wild *H. annuus* ranges from 19.0 — 35.9% with a mean of 25.4%. The polyunsaturated linoleic (18:2) and oleic (18:1) fatty acids varied from 40 — 71% and 13.1 — 40.5%, respectively. Dorrell and Whelan (1978) reported that seed oil varies from 18.1 — 28.6%, averaging 25%. Oleic acid varied from 12.3 — 19.2% averaging 14.8% and linoleic acid varied from 74.7 — 82.6% averaging 79.7%. They also reported that palmitic acid varies from 2.6 — 6.1% averaging 3.5% and stearic acid varies from 1.3 — 2.6% averaging 2.0%. Knowles *et al.*, (1970) reported that palmitic acid reaches a high of 12.9% in California and up to 16.6% in the central plateau of Mexico. They also reported that stearic acid ranges up to 9% and 13% in California and the central plateau of Mexico, respectively.

Environmental factors, especially temperature during the period of seed development and maturation, have been shown to affect oil content and oil composition of maturing cultivated sunflower seed. The effects of temperature on total oil content, however, have been shown to be variable. Robertson *et al.*, (1979) found that latitude and average temperature from the full bloom stage to harvest of field-grown sunflower in North America had no significant effect on total oil content of seed obtained from 22 locations in 1976 or from 35 locations in 1977. However, Canvin (1965) reported that sunflower grown at a constant 21°C had a higher oil content than those grown at either a lower or higher temperature. Harris *et al.*, (1978) showed that total content decreased as temperature increased, whereas Unger and Thompson (1981) showed that oil content decreased as air temperature decreased. These differences in responses may have been due to temperature effects at specific developmental stages or to factors other than temperature. Other factors indirectly related to temperature, such as total solar energy or daylength, may have influenced the total oil content.

Percentages of oleic and linoleic acid of cultivated sunflower oil vary greatly, depending mainly upon the temperature during seed development (Canvin, 1965; Harris *et al.*, 1978; Robertson *et al.*, 1979). Seed maturation during periods of high temperatures produces oil high in oleic acid and low in linoleic acid, compared to seed maturation during periods of low temperatures. Oleic and linoleic comprise 85 to 90% of the oil in sunflower. Palmitic and stearic acids and minor amounts of myristic, palmitoleic, hexadecadienoic, lineolenic, arachidic, and gadoleic acids comprise the remainder of the oil (Unger and Thompson, 1981). Unger and Thompson (1981) found that total solar radiation and daylength were significantly related to oleic and linoleic acid concentration of oil of cultivated sunflower.

Differing environmental effects, especially temperatures, probably affected the fatty acid concentration of the oil of wild populations of *H. annuus* already mentioned since they may have been collected from different locations and environments. The objectives of this study were to 1) observe the chronological development of seed oil and fatty acid concentrations of several indigenous populations of wild *H. annuus* in a uniform environment and 2) compare oil and oil quality of originally collected seed with sibbed and open-pollinated seed of these populations grown under uniform conditions.

MATERIALS AND METHODS

The 38 populations used in this study were collected from wild populations of *H. annuus* throughout the United States from 1977 through 1979. The populations examined were obtained from a region ranging in latitude from 29° to 46°N and in longitude from 81° to 122°W. All populations were planted May 12, 1980, at Bushland, Texas, which is about 35°N latitude and 102°W longitude. Elevation of Bushland is 1180 m and the average dates of first and last frost are 28 October and 18 April, respectively.

Five flowering heads per population per week, in about 50% bloom, were sibbed or inter-pollinated. Sibbing was necessary because of the self-incompatibility of wild *H. annuus*. Heads were bagged and tagged to prevent cross pollination and contamination by other pollen. Some populations completed flowering in as little as 3 weeks while others flowered over a 12-week period. Seed samples were

Table 2. The group means of the agronomic characters for division into 10 groups.

Group no.	Anth. date	Vigor rating	Seed length	Weight 200 seeds	Oil	C18:2	Number of accessions*
		scale 1 to 5	cm	g	%	%	
1	6 — 11	2.6	4.7	1.6	28.6	69.5	40
2	6 — 10	2.7	4.9	1.7	31.6	68.3	22
3	6 — 7	2.1	4.7	1.6	28.4	67.6	30
4	6 — 13	1.7	4.2	1.3	29.4	65.6	3
5	6 — 8	3.9	5.3	2.5	29.6	63.8	8
6	6 — 15	2.3	4.7	1.4	29.2	70.5	7
7	6 — 3	2.1	4.6	1.3	26.2	63.4	12
8	6 — 2	2.7	4.8	1.6	29.3	70.0	36
9	6 — 15	3.7	5.4	2.6	30.2	62.4	3
10	5 — 23	2.6	5.1	2.2	29.9	70.2	11
Pop x	6 — 6	2.5	4.8	1.7	29.0	68.3	177

*5 accessions not included in any group

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VARIABILITY IN OIL CONTENT IN SEED OF *HELIANTHUS* SPP.

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ABSTRACT

Seed samples of a large number of wild sunflower species collected in the U.S.A. in 1980 were analysed for oil content by a NMR analyser. There was a considerable variability both among the species and among different populations of the same species. The analyses, which included all annual and a number of perennial species, found the oil contents to vary between 10 and 40%. The highest oil content, 35% on the average, was found in *H. anomalus* Blake; among the annual species, *H. annuus* L. had the lowest oil content (22%). Some populations of *H. anomalus* had as much as 40% of oil in seed which simplifies their introduction into our breeding program.

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taken 28 days after flowering which was close to physiological maturity. The number of samples per population varied with the length of the flowering period for a population. Open pollinated seeds were collected from all populations at physiological maturity. Oil content was determined on three heads per week per population by nuclear magnetic resonance (NMR) analysis using a 2-ml portion of each seed sample. Each sample was cleaned to remove any empty seeds and dried at 60°C for 24 hours (Granlund and Zimmerman, 1975). Oil was reported on a dry weight basis. The precision of the NMR analyzer with the 2-minute, 11-second integration time was ± 0.1% oil.

Fatty acid composition of solvent-extracted oil was determined by gas chromatography (Zimmer and Zimmerman, 1972). An electronic digital integrator was used to quantify individual fatty acids, which normally accounted for 99+% of sunflower oil. Fatty acid analyses were run on the same samples as the oil analyses.

Temperature and solar radiation were measured at a weather station about 0.5 km from the field. Average maximum, minimum temperature, and mean temperature; total solar radiation; and day-length were recorded for each sampling period. Elevation, latitude, and longitude from the original collection site were recorded for each population.

Seeds from the originally collected populations were analyzed for seed oil and fatty acid composition. These values were then compared to the values obtained from both sibbed and open-pollinated seed of the same population in a uniform environment. Seed samples of the sibbed and open-pollinated populations consisted of subsamples from each week, pooled into one sample for each population. All analyses were replicated twice. Data were analyzed by the analysis of variance, and significant means were separated by Duncan's New Multiple Range Test.

Chronological development of oil and fatty acids was monitored in a commercial sunflower "Hybrid 896", to compare development in cultivated and wild sunflower under approximately the same environmental conditions. Dates of flowering in the commercial hybrid, which reflects the date of planting, were paired with flowering dates for the wild species for comparisons. Not all flowering dates of the wilds were represented by a flowering date in the commercial hybrid, but a representative sample was present.

RESULTS AND DISCUSSION

Oil content across *H. annuus* populations averaged 25.5% and ranged from 17.2% to 34.2%. These values were very close to those reported by Thompson *et al.,* (1981). The chronological development of oil content in seed from different geographic populations grown under a common environment is shown in Table 1. Oil content remained relatively stable from the July 6 sampling through the September 21 sampling. Thereafter, there were slight increases in seed oil content to a maximum of 27.6% for the October 5 sampling. The oil content of wild *H. annuus* populations remained more stable and did not vary by flowering date as much as the oil content did in the commercial hybrid (Table 2). If one evaluates populations of wild *annuus* for oil content, later flowering heads would contain a slightly higher oil content than earlier flowering heads. In reality, when collections are made, a number of heads from earlier flowering are usually mixed with the later flowering ones, thereby giving an average of the older and younger seeds. Seeds from heads which flowered 4 — 6 weeks before usually shatter and are not collected, which may have increased the "average" oil content for some populations.

Table 1. Effect of flowering date on the oil content and fatty acid composition of sunflower seed from 38 indigenous wild *H. annuus* populations.

Date of flowering	No. of samples	Average oil content	Average Fatty Acid Composition			
			Palmitic	Stearic	Oleic	Linoleic
6 July	15	24.8	5.3	2.5	32.1	59.3
13 July	46	24.9	4.9	2.6	29.8	61.9
20 July	74	25.4	4.8	2.4	29.1	62.8
27 July	84	25.5	4.8	2.4	28.0	64.0
3 Aug.	84	25.8	4.8	2.4	28.3	63.7
10 Aug.	99	25.8	5.0	2.5	27.7	64.4
17 Aug.	105	25.5	5.2	2.7	23.5	68.0
23 Aug.	93	25.6	5.2	2.7	23.3	69.4
30 Aug.	89	25.6	5.4	3.0	18.1	73.0
7 Sept.	78	25.3	5.3	3.1	16.5	74.5
15 Sept.	60	25.3	5.4	3.4	15.0	75.5
21 Sept.	30	26.7	5.6	3.7	13.5	76.2
28 Sept.	24	26.6	5.4	3.6	12.8	77.7
5 Oct.	15	27.6	5.5	3.7	13.3	76.9

Table 2. Chronological development of oil content and fatty acids in a commercial Hybrid 896.

Date of flowering	Oil content	Average Fatty Acid Composition			
		Palmitic	Stearic	Oleic	Linoleic
25 June	39.0	4.5	1.8	55.6	37.1
29 June	40.8	5.4	2.2	51.5	39.9
6 July	44.2	5.8	1.8	49.8	41.6
15 July	42.9	5.2	2.0	47.9	43.8
27 July	37.8	4.8	1.9	39.9	53.7
6 Aug.	38.6	5.7	2.3	28.2	62.9
24 Aug.	38.1	5.4	2.4	16.6	75.0
13 Sept.	37.9	5.2	3.5	15.8	74.6

Palmitic and stearic acids averaged 4.9% and 2.7%, respectively, in wild sunflower, and ranged from 3.3 — 7.0% and 1.7 — 5.3%, respectively. Oleic and linoleic averaged 23.3% and 68.3%, respectively, and ranged from 16.1 — 41.9% and 50.8 — 80.4%, respectively. The development of palmitic acid appeared to be cyclic, being high at first, then slightly lower, and reaching a maximum at the last flowering date (Table 1). The development of stearic acid remained at the same level for July 6 — August 23 flowering dates and increased thereater to the highest at the October 5 flowering date (Table 1). Comparing the development of palmitic acid in the commercial hybrid (Table 2) with the development of palmitic acid in the wild populations, the values were quite similar, but there was more of a cyclic trend in the commercial hybrid. Stearic acid followed much the same trend in the hybrid as wild *H. annuus,* in that the highest stearic acid value was obtained at the last sampling.

Oleic and linoleic acids followed opposite developmental patterns. This was true for the wild *H. annuus* and the cultivated sunflower. As oleic acid decreased, linoleic acid increased as evidenced by a high correlation coefficient ($r = -0.96$). It has been hypothesized that oleic acid is transformed to linoleic in sunflower (Hopkins and Chisholm, 1961) which would account for this inverse relationship. The developmental differences between wild sunflower versus commerical hybrid for oleic acid are that the commercial hybrid had a much higher level at the earlier samplings, but decreased to about the same level as the wild sunflower at the last sampling. Linoleic acid was higher in the wild seed than the commercial hybrid at the early samplings, but again increased to about the same levels in both at the last sampling.

Daylength, flowering date, maximum, minimum, and mean temperature had a significant effect on oil content, whereas total solar energy, latitude, longitude, and elevation had no effect on seed oil content. These results were similar to those reported by Unger and Thompson (1981) except for daylength. Daylength, which is directly related with temperature, was highly correlated with mean, minimum, and maximum temperature ($r = 0.97$, $r = 0.97$, $r = 0.96$, $P = 0.001$, respectively). The analysis was done on 38 wild populations, which differed significantly for oil content. This large number of populations, each with its own genetic makeup, would seem to indicate that temperature does effect total oil content as tested by the large number of genotype and flowering date combinations offered by these diverse populations.

Oil quality, especially oleic and linoleic acids, are reported to be affected by environmental conditions. Daylength, flowering date, total solar energy, and maximum, minimum, and mean temperature had a significant effect on palmitic, stearic, oleic, and linoleic acids. Correlations between these environmental factors and fatty acids were determined. Palmitic, stearic, and linoleic acids were all negatively correlated with the environmental factors. Oleic acid was positively correlated with all environmental factors. The highest correlation coefficients were obtained for oleic and linoleic acids. Oleic acid was highly correlated with daylength ($r = 0.82$), but followed closely by maximum, mean, and minimum temperature ($r = 0.81$, $r = 0.81$, $r = 0.80$, $P = 0.001$), respectively. Oleic acid was also positively correlated with total solar energy ($r = 0.74$). Linoleic acid had a high correlation with daylength but negatively ($r = -0.81$). Linoleic acid correlations with temperature and total solar energy were $r = -0.80$ and $r = -0.75$, respectively.

Average oil content and palmitic, stearic, oleic, and linoleic acids for seeds of the original population (variable environments) are compared to seeds of sibbed and open-pollinated populations of the original population grown in a uniform environment in Table 3. There was a significant difference between populations for oil content. There were significant differences between the sibbed and open-pollinated populations in a uniform environment, indicating the differences are genetic. Thompson *et al.*, (1979) reported that the genotype of the maternal parent controlled oil percentage in cultivated sunflower, and that the genotype of the pollen parent had no effect on seed oil content. This question was not addressed in the present study. When evaluating wild population seed for total oil content, evaluation of seed of the population from the original site will not always give a reliable oil content. These seeds should be grown in a uniform environment to reduce environmental effects.

Table 3. Average oil content, palmitic, stearic, oleic, and linoleic acids of seeds of the original populations (variable environments), sibbed and open-pollinated populations of *H. annuus* grown in a uniform environment.

Populations	Oil	Palmitic	Stearic	Oleic	Linoleic
Original	22.3c†	3.0c	1.2c	21.3a	74.7a
Sibbed	25.5a	5.4a	2.7a	23.4a	68.7b
Open-Pollinated	24.1b	4.0b	2.2b	22.0a	71.5b

† Values within a group followed by the same letter are not significantly different at the 5% level (Duncan's New Multiple Range Test).

Palmitic and stearic acids were significantly different for the three populations. These acids may be influenced by the pollen parent and the environmental conditions under which they develop. There were no significant differences between the populations for oleic acid, indicating that this factor was under greater genetical control than environmental control. Linoleic acid was significantly different for the original population, but not the others. Linoleic acid from the sibbed and open-pollinated populations were not significantly different, indicating that when evaluating populations for linoleic acid it would not be necessary to sib each population. Genetic control of linoleic acid was not as great as it appeared to be for oleic acid.

Cautions should be used in evaluating seeds of the original populations for oil quality. Oleic acid was the only acid for which values from the original population did not differ from values of the sibbed and open-pollinated populations. If evaluation is desired for palmitic and stearic acids in a uniform environment, sibbing would be desirable to get an accurate measure of the acids. Sibbing would not be necessary for evaluation of oleic and linoleic acids because their values are not significantly different from open-pollinated populations.

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VARIABILITY IN THE COMPOSITION OF HIGHER FATTY ACIDS IN OIL OF SUNFLOWER INBREDS WITH DIFFERENT OIL CONTENTS IN SEED.

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ABSTRACT

When developing sunflower hybrids, oil quality is as important as high oil content in seed. To develop hybrids with different compositions of higher fatty acids (HFAs) in oil, we examined a number of inbreds which varied in the oil content in seed from 22 to 57%. The following conclusions were drawn. The examined inbreds varied significantly in the composition of HFAs. Environmental factors caused variations in the expression of HFAs among the inbreds and within them. A limited number of the inbreds remained virtually unaffected by the environment. The contents of the most important HFAs, linoleic (18:2) and oleic (18:1), displayed the variability necessary for the development of hybrids with different oil quality. Stearic acid (18:0) displayed the largest variability. Myristoleic acid (14:1) was found in a small number of the inbreds only in 1978. The oil content in seed was significantly correlated only with linoleic (positive) and oleic acid (negative). Some correlations were found between the contents of certain HFAs. The correlations were mostly negative.

INTRODUCTION

When developing sunflower hybrids on the basis of male sterility, one should breed for both, high oil content in seed and certain oil quality. The biological value of oil is estimated by the contents of HFAs and liposoluble vitamins paying special attention to the contents of essential fatty acids (EFA) and alpha-tocopherol, i.e., vitamin E. Oleic (18:1) and linoleic acid (18:2) are the most important HFAs.

Ivanov (1974), Konstantinov *et al.*, (1974) and other authors found differences in the composition of HFAs in various sunflower genotypes and perceived the effect of environmental factors on their expression. Fernandez-Martinez and Knowles (1976) found large variability in the composition of HFAs in wild sunflowers. According to Skoric *et al.*, (1978), the inheritance of oleic and linoleic acid in F1 is controlled by the nonadditive component of genetic variability.

Although linoleic acid commonly prevails over oleic acid in sunflower oil, the market frequently demands oil with a high content of oleic acid. Soldatov (1976) pointed at the possibility of using mutagenic chemicals to develop a high oleic sunflower variety. His variety Pervenec, which has a high content of oleic acid in oil, could be used in breeding to change FA composition in future sunflower hybrids.

MATERIALS AND METHODS

The subject-matter of this paper is a study conducted during 1978 — 1980 to determine the variability in the composition of HFAs in oil of different inbreds and the effect of environmental factors on the expression of HFAs. We analysed 86 inbreds beyond S7, of different genetic origins, with known GCA for the important agronomic characters, which were grown in field in 1978 and 1979 under uniform cultural practices. Average seed samples were analysed for oil content in seed by the method of NMR and for the content of HFAs in oil by the method of gas chromatography. The graphs enclosed show the contents of several HFAs; the other will only be discussed.

In 1980 we examined 16 inbreds varying in their oil contents and composition of HFAs. The data were processed by the regression analysis and correlation coefficients were calculated between oil content in seed and contents of HFAs and between contents of individual HFAs.

RESULTS AND DISCUSSION

The oil contents in seed ranged from 22 to 57%. Environmental factors affected largely the final oil content as well as the processes of oil formation and the accumulation of individual HFAs in oil.

The examined inbreds differed in the content of oil and the composition of HFAs in oil. The variability in linoleic acid (18:2) depended on genotype and environmental factors. The latter were less favorable in 1978 than in 1979; consequently, the contents of linoleic acid ranged from 60 to 67% in 1978 and from 66 to 70% in 1979 (Figure 1).

As there was a highly significant negative correlation between the contents of linoleic (18:2) and oleic acid (18:1) ($r = -94$), environmental factors had the contrary effect on the expression of oleic acid (Figure 2). Most of the inbreds had a higher content of oleic acid in 1979 than in 1978.