Wild sunflower species from the southeastern United States as potential sources for improving oil content and quality in cultivated sunflower

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ABSTRACT

Sunflower (*Helianthus annuus* L.) oil has the potential to be improved for nutritional and industrial purposes through selection and breeding. The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from wild species, resulting in a continuous improvement in agronomic traits. Interest in using wild species in breeding programs has increased, but information about oil concentration and fatty acid composition is lacking for a number of rare and threatened species. The objective of this study was to evaluate achenes of seven wild sunflower species, *H. eggertii*, *H. schweinitzii*, *H. porteri*, *H. verticillatus*, *H. smithii*, *H. angustifolius*, and *H. atrorubens*, from the southeastern USA for oil concentration and fatty acid composition of four major fatty acids, palmitic, stearic, oleic and linoleic acids. *Helianthus verticillatus* had the highest oil concentration of any species with 324 g/kg and was within the range expected for a wild perennial sunflower species. The high linoleic acid concentration in *H. porteri* of 815 g/kg is the highest concentration reported for a wild sunflower species. Linoleic acid concentrations for all seven species were higher than expected for populations grown in southern latitudes. The lower saturated fatty acid profile in several of the species indicates these species have the potential to reduce saturated fatty acids in commercial sunflower oil. Further research will be needed to determine the inheritance of the fatty acids and oil concentration. Other agronomic traits will need to be maintained during the introgression of these traits into sunflower.

Key words: *Helianthus* – linoleic acid- oil – oleic acid – palmitic acid – stearic acid.

INTRODUCTION

Vascular plants produce many compounds and secondary metabolites, one of which is oil resulting from plant lipid synthesis. Although oil concentrations of up to 37 g/kg have been reported in whole plants of wild sunflower (*Helianthus annuus* L.), the achenes are the primary storage tissue for oil (Seiler et al., 1990). The oil that accumulates in the achenes of wild and cultivated sunflower is composed of triacylglycerols that exist in the liquid form at room temperature and have a low melting point. The fatty acid composition of the achene oil determines its end use suitability. Sunflower oil is currently considered high quality edible oil; however, the potential for improved nutritional and industrial characteristics through selection and breeding has not been exhausted.

Since the development of high-oleic sunflower hybrids, sunflower oil has become a more important feedstock for the oleochemical industry, of which the cosmetics industry is a major user. Development of the mid-oleic NuSun® oil in the USA has increased the demand for this type of oil in the food processing industry due to its high oxidative stability (Kleingartner, 2002).

Oil concentration and fatty acid composition, especially oleic and linoleic fatty acids, of oil from wild and cultivated sunflower varies greatly mainly as a response to temperature during seed development (Harris et al., 1978; Seiler, 1986). A high temperature during seed maturation results in oil with high oleic acid concentration, and a low linoleic acid concentration. Generally, the cooler northern latitudes produce considerably higher concentrations of linoleic acid in the oil than the warmer southern latitudes (De Haro and Fernández-Martínez, 1991).

The genus *Helianthus* consists of 51 species and 19 subspecies with 14 annual and 37 perennial species (Schilling, 2006). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from wild species. This has resulted in continuous improvement of agronomic and economic traits in cultivated sunflower (Thompson et al., 1981; Seiler, 1992; Seiler and Rieseberg, 1997; Jan and Seiler, 2007). Recent emphasis on the oil concentration and fatty acid composition of sunflower achenes has increased interest in using wild species in breeding programs to enhance oleic or linoleic acid, or to reduce saturated fatty acids.

While a few populations of some wild sunflowers have been collected and evaluated for oil concentration and fatty acid composition, many remain to be evaluated to fully characterize the available genetic diversity. There is an urgent need to collect and evaluate species that are endemic to limited geographic areas and may be at risk of being eliminated by the activities of man.

The objective of this study was to evaluate achenes of seven wild sunflower species, *H. eggertii*, *H. schweinitzii*, *H. porteri*, *H. verticillatus*, *H. smithii*, *H. angustifolius*, and *H. atrorubens* collected from the southeastern region of the USA for oil concentration and fatty acid composition of four major fatty acids, palmitic, stearic, oleic and linoleic acids.

MATERIALS AND METHODS

Plant materials. Populations of wild sunflowers were collected between 17 and 28 October, 2003. The expedition covered a distance of 4600 km in five states: Alabama, Georgia, North Carolina, South Carolina, and Tennessee. Details of this exploration can be found in Gulya et al. (2007). Heads of wild sunflowers were collected from 50 to 250 plants within each population and bulked into a single sample. Herbarium specimens were deposited at the USDA-ARS wild *Helianthus* herbarium at Fargo, North Dakota. Achene samples were sent to the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, USA where they are maintained and distributed.

All populations were collected throughout the broad distributional range of the species. Prior collection sites obtained from herbarium specimens and generalized distribution maps were used to locate populations. Population size (number and extent), habitat, soil type, achene set per head, the presence of diseases and insects, GPS coordinates including elevation, and the presence of other wild sunflower species located near the collection sites were recorded for each population.

Oil and fatty acid analyses. Achenes were stored at 5°C and low humidity (<20%) until analyzed. Each sample represented an isolated, open-pollinated segregating population. Two 6-ml portions from each achene sample were cleaned to remove empty achenes, and analyzed for oil concentration (expressed as a percent on a dry weight basis) by nuclear magnetic resonance (Granlund and Zimmerman, 1975). Fatty acid composition was determined from a 10- to 20-achene sample. A small portion of the pulverized sample (10 to 20 mg) was transferred to a disposable filter column (Fisher Scientific[1]) and eluted with 3.5 ml of diethyl ether. The oil in the diethyl ether solution was converted to methyl esters using an organic-catalyzed transesterification technique (Metcalfe and Wang, 1981). The sample was injected into a Hewlett-Packard 5890 gas chromatograph containing a DB-23 capillary column (25 m x 0.25 mm, J & W Scientific). A fatty acid standard, 21A (Nu-Chek-Prep, Inc.), containing methyl esters of the following acids was used as a reference: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), arachidic (20:0), 11-eicosenoic (20:1), behenic (22:0), and lignoceric (24:0). Fatty acid and oil concentrations were means of two samples per population.

Data analysis. The data were analyzed using an analysis of variance (ANOVA). Means were separated using Duncan's New Multiple Range Test.

RESULTS

Achenes were collected for five rare species, *H. eggertii* from Alabama, South Carolina, and Tennessee, *H. schweinitzii* from North and South Carolina, *H. porteri* from North Carolina and Georgia, *H. verticillatus* from Alabama and Tennessee, and *H. smithii* from North Carolina (Table 1). Two additional species with wider distributional ranges, *H. angustifolius* from Georgia and Tennessee, and *H. atrorubens* from Alabama were also collected.

Helianthus porteri is an annual species that was recently transferred from the genus *Viguiera* (Pruski 1998). Eight populations of this species were collected from its known distributional range (Table 1). The average oil concentration was 288.7 g/kg (Table 2).

Among the seven species, the lowest average oleic acid concentration of 65.2 g/kg was observed in *H. porteri* (Table 2). This was accompanied by a high linoleic acid concentration. The 814.9 g/kg average linoleic concentration in *H. porteri* was the highest observed in any wild species, with one population having a concentration of 830 g/kg. The concentration of palmitic and stearic acids in *H. porteri* averaged 55.8 g/kg and 32.1 g/kg, respectively.

Table 1. Wild *Helianthus* populations analyzed for oil concentration and fatty acid composition of seven species collected from the southeastern USA

Species	Number of populations	State (Location)	Habitat
ANNUAL			
H. porteri	8	Georgia and North Carolina	Granite outcrops, Piedmont Region
PERENNIAL			
H. angustifolius	2	Georgia and Tennessee	Open to shaded woods, usually moist places
H. atrorubens	1	Alabama	Open mixed woods, dry roadsides, dry shaded hillsides
H. eggertii	13	Alabama, South Carolina, and Tennessee	Grassy openings, barrens, open oak-hickory woods
H. schweinitzii	14	North and South Carolina	Open woodlands, clearings, Piedmont region
H. smithii	1	North Carolina	Dry, open woods
H. verticillatus	2	Alabama and Tennessee	Moist, prairie-like openings, and edge of woodlands, clay soil

Helianthus eggertii is a hexaploid perennial species that was recently removed from the threatened species list by the U.S Fish and Wildlife Service (USFWS 2005). Thirteen populations were collected from all areas of the species range, except from Kentucky. Oil concentration of the populations averaged 285 g/kg, while oleic and linoleic acids averaged 154.6 and 721.4 g/kg, respectively (Table 2). The saturated fatty acids, palmitic and stearic acids averaged 55.6 and 28.4 g/kg, respectively.

Helianthus schweinitzii is a federally protected rare hexaploid species in the Piedmont region of North and South Carolina. Fourteen populations of this species were collected from throughout its range. The oil concentration of the 14 populations averaged 285 g/kg, the same as *H. eggertii* (Table 2). Saturated palmitic and stearic acid concentrations averaged 53.6 and 39.0 g/kg, respectively, while oleic and linoleic acids averaged 108.7 and 765.2 g/kg, respectively.

Helianthus smithii, a rare diploid perennial species, was collected from a single population in North Carolina, near the eastern edge of its distributional range. The oil concentration was 296 g/kg, while oleic and linoleic acid concentrations were 104.2 and 778.4 g/kg, respectively (Table 2). Palmitic and stearic acid concentrations were 50.7 and 32.7 g/kg, respectively.

Perennial *H. verticillatus* is a species which was described over 100 years ago (Small, 1898), but was not rediscovered or recollected until recently in Tennessee (Mathews et al., 2002). Two populations of this species were collected, one from Tennessee and one from Alabama. Oil concentration averaged 323.5 g/kg, the highest concentration of any species analyzed in the study (Table 2). The saturated fatty acids, palmitic and stearic, averaged 57.6 and 25.8 g/kg, respectively, while oleic acid and linoleic acids averaged 137.6 and 744.3 g/kg, respectively.

Oil concentration of one population of diploid perennial *H. atrorubens* and two populations of diploid perennial *H. angustifolius* averaged 277 and 297.5 g/kg (Table 2). Oleic acid and linoleic acids averaged 89.6 and 778.5 g/kg for *H. atrorubens* and 117.2 and 723.1 g/kg for *H. angustifolius*, respectively. Palmitic and stearic acids averaged 63.4 and 34.1 g/kg for *H. atrorubens* and 74.0 and 37.9 g/kg for *H. angustifolius*, respectively.

DISCUSSION

Wild *Helianthus* populations generally have oil concentrations of 250 to 300 g/kg, much lower than 450 to 500 g/kg in cultivated sunflower (Seiler, 1985; 1994). In one study, wild *H. annuus* averaged 258 g/kg and *H. petiolaris* 288 g/kg (Seiler, 1983). The oil concentration for the perennial sunflowers from Canada averaged 297 g/kg (Seiler, 1999) compared to 244 g/kg for perennial species from the central Great Plains of the USA (Seiler, 1994).

Table 2. Oil concentration (g/kg) and fatty acid composition (g/kg) of seven *Helianthus* species collected from the southeastern USA

Species	Oil concentration	Fatty acid composition[1]			
		Palmitic	Stearic	Oleic	Linoleic
ANNUAL					
H. porteri	288.7±19.1 NS	55.8±3.9ab[1] *	32.1±4.4ac **	65.2±11.0d **	814.9±13.3a **
PERENNIAL					
H. angustifolius	297.5±21.9 NS	74.0±0.8b *	37.9±0.1ab **	117.2±24.3bc **	723.1±42.0b **
H. atrorubens	277.0±0.0 NS	63.4±0.0ab *	34.1±0.0a-c **	89.6±0.0cd **	778.5±0.0ab **
H. eggertii	285.0±29.6 NS	55.6±16.2ab *	28.4±3.6bc **	154.6±17.6a **	721.4±48.7b **
H. schweinitzii	285.0±28.7 NS	53.6±4.1b *	39.0±6.3a **	108.7±13.0bc **	765.2±13.7ab **
H. smithii	296.0±0.0 NS	50.7±0.0b *	32.7±0.0a-c **	104.2±0.0c **	778.4±0.0ab **
H. verticillatus	323.5±27.6 NS	57.6±2.1ab *	25.8±2.0c **	137.6±26.1ab **	744.3±23.0b **

[1]Means followed by the same letter in a column are not significantly different at the P<0.05* and P<0.01** level according to the Duncan's New Multiple Range Test.

One previous report of oil concentration for a single population of *H. porteri* from Georgia was 174 g/kg (Seiler, 1985). This low concentration compared to the 288.7 g/kg concentration in the current study may be due to the achene quality or the one population examined was not representative of the species. The oil content of *H. eggertii* in this study was 285 g/kg, higher than the 222 g/kg concentration reported by Thompson et al. (1981). Achenes from the original populations were evaluated in both studies. The higher oil concentrations observed in the current study may be more representative of the potential genetic diversity of the species than the concentration reported by Thompson et al. (1981) because of the larger number of populations analyzed in the current study. A previous report of oil concentration in *H. schweinitzii* was only 82 g/kg, an extremely low value resulting from a very low achene weight, which may indicate incomplete achene filling (Seiler, 1985). The oil concentration of 285 g/kg in the current study is closer to that normally observed for a perennial sunflower species from this region. The reported oil concentration for *H. smithii* of 296 g/kg from a single population is within the high range of 247 to 296 g/kg previously reported for this species (Seiler, 1985).

Helianthus verticillatus had the highest average oil concentration of any species in the current study. This is the first report of oil concentration and fatty acid composition of this recently rediscovered species. This species is restricted to three sites, two of which were collected for this study. The mean oil concentration in *H. angustifolius* in the current study was 297.5 g/kg, slightly higher than the 252 g/kg

reported for the single population evaluated by Thompson et al. (1981). The few populations evaluated probably do not represent the total genetic variability since only a small portion of its distributional range was sampled. The oil concentration of *H. atrorubens* averaged 277 g/kg, which is within the range of 211 to 320 g/kg based on 15 populations reported by Seiler (1985).

Thompson et al. (1981) concluded that the environments (geographic location) in which collections of different species were made did not influence the oil percentage. The effect of temperature on oil content in sunflower has been shown to be variable. Seiler (1983) showed that environmental factors related to temperature are not related to oil concentration in wild *H. annuus*. Robertson et al. (1979) found that average temperature during full-bloom to harvest stages of field-grown sunflower in North America had no significant effect on oil content of seed obtained from 22 locations in 1976 or 35 locations in 1977. Harris (1978) concluded that oil content decreased as temperature increased; whereas Unger and Thompson (1982) observed a decrease in oil content as temperature decreased.

Oil concentration of interspecific hybrids can be rapidly increased to an acceptable level by backcrossing with cultivated sunflower lines (Seiler and Rieseberg, 1997). Based on this fact, there should be little concern about the lower oil concentration of the wild species when they are used as sources of genes for unique traits.

The 814.9 g/kg linoleic acid concentration for *H. porteri* is the highest of any sunflower species. Previous reports for linoleic acid for this species were 834 g/kg in one population from Georgia (Seiler, 1985) and one population regenerated in France with 818 g/kg (De Haro and Fernández-Martínez, 1991).

The fact that populations of all seven species had linoleic concentrations > 720 g/kg indicates that this trait should have a genetic basis because it is relatively stable in the different populations and species over a wide range of environments. High linoleic sunflower oil with > 700 g/kg is preferred for the production of soft margarine (De Haro and Fernández-Martínez, 1991).

The linoleic fatty acid concentration observed in the *H. porteri* populations and other rare species was unusually high for southern latitudes. High temperatures during flowering, achene filling, and maturation favor a low linoleic acid concentration and a high oleic acid concentration (Seiler, 1986). Generally, the cooler northern latitudes have higher concentrations of linoleic acid in the oil than the warmer southern latitudes (De Haro and Fernández-Martínez, 1991). A lower concentration of 540 g/kg of linoleic acid is more typical of the concentration expected in warmer southern latitudes.

There is a strong negative relationship between linoleic and oleic acid concentrations; i.e., if linoleic increases, oleic decreases (Seiler, 1983; 1986). This relationship is common to both wild and cultivated sunflower. Thus, the high linoleic concentration of 818 g/kg in *H. porteri* is accompanied by a corresponding low concentration of oleic acid near 65 g/kg. Low oleic acid concentrations for *H. porteri* from Georgia, USA were reported by Seiler (1985) with 55 g/kg and by De Haro and Fernández-Martínez (1991) with 60 g/kg for one population grown in France. Reports of oleic concentrations from other populations of *H. eggertii* were 210 g/kg compared to 285 g/kg in the current study (Thompson et al., 1981). For *H. smithii*, it was 190 g/kg for a single population grown in Spain, and 180 g/kg when grown in France (De Haro and Fernández-Martínez, 1991).

The environmental relations between saturated palmitic and stearic fatty acids are less clear than those for linoleic and oleic fatty acids. In a study based on a few wild sunflower species, those collected from northern latitudes had lower saturated fatty acids than those from further south (Seiler, 1994; 1999). The mean palmitic acid concentration ranged from 50.7 g/kg for *H. smithii* to 74 g/kg in *H. angustifolius*, while stearic acid ranged from 25.8 g/kg in *H. verticillatus* to 39 g/kg in *H. schweinitzii*. This is similar to wild *H. annuus* with 51 g/kg palmitic and 31 g/kg stearic acid (Seiler, 1983). The combined palmitic and stearic acid concentration ranged from 83.4 g/kg in *H. smithii* and *H. verticillatus* to a high of 111.9 g/kg in *H. angustifolius*. The saturated palmitic and stearic fatty acids totaling 83 to 88 g/kg are about 30 % less than typical cultivated sunflower oil with approximately 120 g/kg. Lower saturated fatty acids in sunflower oil may be possible by using these species.

CONCLUSIONS

The introgression of wild species into cultivated sunflower with different fatty acid profiles and a stable linoleic concentration could facilitate the expansion of commercial sunflower production into the southern latitudes. There appears to be sufficient variability to introduce and select for high linoleic acid concentration and reduced saturated fatty acid concentrations in cultivated sunflower oil. Further research will be needed to determine the inheritance of fatty acid composition and oil concentration. Other agronomic traits will need to be maintained during the introgression of these traits into cultivated sunflower. The addition of these wild species populations to the wild sunflower germplasm collection

significantly increases the available genetic diversity for improving cultivated sunflower and also insures their future preservation.

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