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Whole-plant Utilization of Sunflowers

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ABSTRACT

Forty-eight accessions representing 39 taxa of the genus Helianthus *(sunflower) were collected from a common garden and whole plants examined for yields of non-polar extractables (hydrocarbons, rubber, etc.), polar extractables (resins, sugars, etc.) and crude protein. This study revealed several promising species for obtaining hydrocarbons:* H. agrestis, H. annuus, H. argophyllus, *and* H. arizonensis. *Species highest in methanol extractables were* H. ciliaris, H. leavigatus, H. occidentalis *ssp.* plantagineus, H. paradoxus, H. petiolaris, *and* H. silphioides. *Species with greater than 16% protein yields were* H. arizonensis, H. grosseserratus, H. neglectus, H. petiolaris *ssp.* fallax, *and* H. simulans. *Since this genus is well known agronomically, the development of one of the species for whole-plant utilization may be easier than the domestication of some lesser-known genera.*

INTRODUCTION

The sunflowers (*Helianthus*) have been developed into an important agricultural crop.[1] With the decline in world supplies of hydrocarbons, there has been a particular interest in the direct use of oils (triglycerides) of sunflower seed as diesel fuel,[2,3] and in catalytic conversion to other liquid fuels.[4,5] The reports on the analyses of 200 species of plants for whole-plant hydrocarbons and rubber[6,7] have shown several promising species for possible hydrocarbon farming in the future.[8] However, whole-plant utilization, coupled with a multi-product scenario, appears to be very promising in sunflowers.

Phytochemicals. Phytochemical studies on *Helianthus* species to date have led to the isolation and characterization of acetylenes,[9–12] flavonoids,[13,14] sesquiterpenoids,[9,11,13–19] diterpenoids,[11,13,14,18,20,21] and natural rubber.[21,22] Some of the diterpene carboxylic acids and sesquiterpene lactones present in *Helianthus* species have been demonstrated to possess plant growth regulator[16,17,23] and insecticidal activities.[14,24] Structurally related sesquiterpene lactones from phylogenetically related taxa have been shown to exhibit antimicrobial activity against Gram-positive bacteria.[25] It is believed[14,24] that certain diterpene carboxylic acids and sesquiterpene lactones may contribute to the natural resistance of *Helianthus* species to insect predation.[26,27]

Rubber. Although sunflowers have not been completely screened for natural rubber, Swanson *et al.*[28] reported that *H. hirsutus* contains natural rubber with a molecular weight of $2{\cdot}79 \times 10^5$ and polydispersity factor (3·1) which indicates a potential as a source of natural rubber. A more extensive study of 53 *Helianthus* taxa found rubber concentration from a trace to 1·89% in the leaves.[21,22] Sampling of both stems and leaves indicates that the rubber is found primarily in the leaves rather than in the stems, in contrast to guayule (*Parthenium argentatum*).[21,22] *Helianthus annuus* originating from Winton, Oklahoma, had 1·45% rubber.[21] The same species from Tucson, Arizona, contained 0·55%[29] and a sample collected in Ontario, Canada, contained only 0·26% rubber.[30] This could be evidence of environmental effects or of genetic variation within species.

Speciality carbohydrates. The sunflower may also be a promising source of commercial pectin. The mature head of sunflower cultivars grown in western Canada contains 3·3–5% of high-ester pectin and 11·8–14·3% of low-ester pectin.[31,32] The level of pectin which might be found in the leaves or stems of other *Helianthus* species is as yet unknown. The Jerusalem artichoke (*H. tuberosa*) and its hybrid with

H. annuus are used world-wide as a source of food. The tubers are rich in starch and sugars, and are being investigated as a biomass crop for ethanol production. They also contain inulin, which is converted in the body to fructose, a sugar which is suitable for use by diabetics. It is also a commercial source of fructose.[1,33]

Livestock feed. In addition to phytochemicals, natural rubber, and speciality carbohydrates, the extracted residue may be utilized as an animal feed. Not only are sunflowers devoid of any known toxins for higher animals, but they have been used extensively on a whole-plant basis as an animal forage.[34] Sunflowers were reintroduced to the United States from Russia as a silage crop recommended for planting on the fringes of the corn belt where they would be more productive than corn. Marx[34] compared sunflower silage with alfalfa haylage, and found the sunflower product to contain 11% crude protein, 33·5% crude fiber, 7% ether extract, and 9·5% ash, of which 0·83% was calcium and 0·32% phosphorus. In feeding trials, dairy steers gained 92% as much weight when fed on sunflower silage as with alfalfa haylage.[34]

The taxonomy and evolution of *Helianthus* have been studied extensively by Charles Heiser.[1] About 100 species of *Helianthus* are known, 50 of them native to North America and 15 to South America.[1,35] The genus includes both annuals and perennials, with considerable natural hybridization.[1] Most species have a chromosome number of $n = 17$. *Tithonia* (Mexican sunflower) is a closely related genus with 11 species, four of which have a haploid number of 17.

The USDA Conservation and Production Research Laboratory at Bushland, Texas, maintains a collection of North American species of *Helianthus* in a common garden. Thus, an unusual opportunity was available to examine many of the North American species for hydrocarbon and polar extractable yields, as well as the crude protein yield of the extracted residue.

EXPERIMENTAL

All plants were collected at the USDA Conservation and Production Research Laboratory, Bushland, Texas, in August 1980. Whole aboveground plants were collected to obtain a representative sample. In some cases, several accessions were cultivated and samples were taken from each accession to explore infraspecific variation. All plants are permanently tagged in the common garden and vouchers are on deposit at the Bushland laboratory.

Plant material was dried for 48 h at 70°C and ground to pass a 20-mesh screen. The non-polar hydrocarbons were extracted with cyclohexane in a Soxhlet for 20 h,[36] after which the marc was dried for 3 h at 100°C to remove all the cyclohexane. The material was then extracted for 20 h with methanol to remove polar components (phenolics, sugars, free amino acids, etc.). The cyclohexane extracts were dried at 100°C for 24 h and the methanol extracts dried at 100°C for 48 h. The methanol extracts contained some water used in cleaning out the material in the flasks and thus had to be dried longer to ensure dryness.[36] The residue (marc) was dried at 100°C for 48 h. After drying, all pans were placed in a desiccator for 4 h to allow temperature and humidity stabilization before weighing. Percent yields were calculated as 100 times the weight of the extract divided by the sum of the weight of the extracts and residue. Protein was determined by standard Kjeldahl N × 6·25 at Colorado State University. Principal component analysis (PCA) follows the programs of Blackrith and Reyment[37] and Veldman.[38]

RESULTS AND DISCUSSION

The results from this survey are shown in Table 1. Taxa with the highest yields of non-polar material are *H. agrestis* (7·38%), *H. annuus* from Winton, Oklahoma (7·09%), *H. argophyllus* (6·52%), *H. arizonensis* (6·13%), *H. anomalus* (5·74%), *H. ciliaris* (5·26%), *H. nuttallii* (5·25%) from Orovada, Nevada, *H. praecox* ssp. *hirtus* (5·19%), and *H. nuttallii* (5·17%) from Payson, Utah. Of these eight taxa, five are annuals. Since only 11 annual taxa were present in the 39 taxa examined, there does seem to be a tendency for the annuals to have more cyclohexane extractables. One of the most interesting comparisons is between *H. annuus* (Winton, 7·09%) and *H. annuus* (a commercial hybrid grown at Bushland, Texas, 2·23%). This suggests that there may be considerable infraspecific variation which could be exploited.

Methanol extractables varied from nearly 6·87% to 21%. The highest yielding taxa were *H. petiolaris* spp. *petiolaris* (21%), *H. paradoxus*

(19·54%), *H. occidentalis* ssp. *plantagineus* (18·33%), *H. laevigatus* (18·24%), *H. silphioides* (18·01%), and *H. ciliaris* (17·17%).

Total extractables varied from 8·59% to 23·0%. The largest total yields were obtained from *H. paradoxus* (23·0%), *H. petiolaris* ssp. *petiolaris* (22·86%), *H. ciliaris* (22·43%), *H. laevigatus* (21·77%), *H. agrestis* (20·83%).

In general, those taxa with higher yields are comparable to several promising species (Table 2) previously reported.[36] Non-polar extractable yields greater than 7% are not too uncommon[15] nor are methanol extract yields greater than 18%.

As previously mentioned, rubber has been reported in *Helianthus*[21,22,28] and the average molecular weight (MW) of rubber from *H. hirsutus* has been reported[28] as 279 000 with a molecular weight distribution (MWD) of 3·1. *Hevea brasiliensis* rubber has a MW of about 1 300 000 and MWD of 5·2. If rubber of molecular weight greater than 20 000 can be utilized,[28] the sunflowers might present a source for both phytochemicals and rubber.

Although a thorough examination of sunflower speciality carbohydrates such as pectin[31,32] and inulin is beyond the scope of this paper, these added-value products should be considered when selections are made for future whole-plant utilization. As previously mentioned, sunflower silage has long been considered as a livestock feed.[34] In a feeding trial (dairy steers), Marx[34] used sunflower silage (11·1% crude protein, 6·8% digestible protein) in comparison with alfalfa haylage (18·3% crude protein, 11·9% digestible protein) and got a 92% efficiency of weight gain. Although many factors such as amino acid composition and rumen digestibility will need to be analyzed for the most promising species, we felt it was important to examine the crude protein in the extracted residue in order to determine the natural variation expressed and to help focus research on the most promising taxa. Protein was analyzed in each sample residue (after extraction with cyclohexane and methanol) and is reported in Table 1. A feed with a protein content of 16% is normally required for dairy cattle feed. Several of the samples had greater than 16% protein content: *Helianthus grosseserratus*, 20·1; *H. arizonensis*, 18·4; *H. simulans*, 18·1; *H. petiolaris* ssp. *fallax*, 17·3; and *H. neglectus*, 16·2.

Most of the species that are high in protein are not high yielding for hydrocarbons, rubber or polar extractables. However, it is not obvious

TABLE 1
Analyses of 39 Taxa of *Helianthus*, Representing 49 Accessions

Helianthus species	*Annual (A) or perennial (P)*	*Originally from*	*C-Hex frac.*	*Rubber yield*[a]	*MeOH frac.*	*Total*	*Percent protein*[b]
agrestis	A	Brandenton, FL	7·38	1·66	13·45	20·83	6·9
angustifolius	P	Alvin, TX	3·33	0·18	9·58	12·91	15·9
annuus	A	Winton, OK	7·09	1·40[c]	11·73	18·82	8·7
annuus, hybrid 894	A	Bushland, TX	2·23	0·49	14·65	16·88	8·6
anomalus	A	Mexican Water, AZ	5·74	0·18	12·30	18·04	9·8
argophyllus	A	Rockport, TX	6·52	1·14[c]	9·60	16·12	11·9
arizonensis	P	Snowflake, AZ	6·13	0·28[c]	13·16	19·29	18·4
californicus	P	Napa, CA	3·05	1·78[c]	12·44	15·49	13·8
ciliaris	P	Bushland, TX	5·26	0·57	17·17	22·43	15·6
debilis	A	Titusville, FL	1·95	0·68	8·83	10·78	9·6
deserticola	A	Leeds, UT	3·16	0·82	10·96	14·12	5·3
divaricatus	P	Wister, OK	1·90	0·47[d]	11·54	13·44	2·6
glaucophyllus	P	Blowing Rock, NC	3·29	0·25	9·50	12·79	8·1
grosseserratus	P	Cherokee Co., KS	2·36	0·28	12·28	14·64	14·6
grosseserratus	P	Hooker Co., KS	4·41	0·28	14·37	18·78	20·1
grosseserratus	P	Stuart, OK	3·56	0·28	10·49	14·05	17·1
hirsutus	P	Wilburton, OK	1·60	0·30	8·30	9·90	6·1
laciniatus	P	Mimbres River, NM	3·15	0·31	12·40	15·55	9·9
laetiflorus	P	Lyon Co., KS	2·22	0·66	10·64	12·86	11·9
laevigatus	P	Botetourt Co., VA	3·53	—	18·24	21·77	13·9
maximiliani	P	Bloomington, IN	3·10	—	13·21	16·31	10·8
maximiliani	P	San Jon, NM	3·50	0·24	9·87	13·37	15·3
maximiliani	P	Gatesville, TX	2·53	—	10·30	12·83	8·9
microcephalus	P	Cherokee Co., SC	4·77	0·26[c]	14·25	19·02	14·1

mollis	P	Greenwood Co., KS	3·26	0·31	11·05	14·31	8·9
mollis	P	Okmulgee Co., OK	2·60	0·31	9·72	12·32	8·5
mollis	P	Rivercrest, TX	1·87	0·31	8·58	10·45	6·6
neglectus	A	Kermit, TX	3·83	0·10	11·71	15·54	16·2
nuttallii	P	Orovada, NV	5·25	0·96[c]	10·23	15·48	8·8
nuttallii	P	Payson, UT	5·17	–	12·76	17·93	10·6
occidentalis	P	Raymondville, MO	2·12	0·48	15·14	17·26	11·9
occidentalis ssp. *plantagineus*	P	Sheridan, TX	2·36	1·62	18·33	20·69	8·8
paradoxus	A	Ft. Stockton, TX	3·46	0·15	19·54	23·00	13·3
petiolaris ssp. *fallax*	A	Adrian, TX	2·15	0·30	11·99	14·14	17·3
petiolaris ssp. *petiolaris*	A	Memphis, TX	1·86	0·14	21·00	22·86	12·1
praecox ssp. *hirtus*	A	Carrizo Springs, TX	5·19	0·49	10·05	15·24	13·8
pumilis	P	Boulder, CO	1·72	0·53	6·87	8·59	7·4
resinosus	P	Collins, MS	2·89	1·78[c]	11·76	14·65	11·9
rigidus ssp. *rigidus*	P	Brookston, IN	1·86	–	9·93	11·79	7·8
rigidus ssp. *subrhomboides*	P	Leyden, CO	1·42	–	10·90	12·32	9·9
salicifolius	P	Kansas	3·13	0·37	9·30	12·43	7·1
salicifolius	P	Muenster, TX	3·26	0·37	9·31	12·57	11·2
silphioides	P	Wister, OK	2·63	0·42	18·01	20·64	10·0
simulans	P	Milton, FL	3·42	0·31	13·91	17·33	18·1
smithii	P	Morgantown, NC	4·48	0·58[c]	11·77	16·25	12·2
strumosus	P	Siler City, NC	2·98	0·55	11·80	14·78	12·9
tuberosus	P	Kilgore, TX	2·26	0·93	13·28	15·54	12·1
tuberosus x *annuus*	P	Turlock, CA	1·73	–	12·21	13·94	9·3
		Average	3·39	0·57	12·26	15·65	11·35

[a] Rubber yields, for leaves except for *divaricatus*, are from Stipanovic *et al.*[21,22]
[b] Protein determined by Kjeldahl N x 6·25.
[c] By ^{13}C-NMR spectral analysis. All others by gravimetric.
[d] Whole plant analyzed.

TABLE 2
Comparison of the Yields of Extractables for some Sunflowers and High Yielding Species Previously Examined[36]

Plant species	*Cyclohexane % yield*	*Methanol % yield*	*Total yield*
Asclepias speciosa	4·72	18·67	23·39
Baccharus neglecta	5·20	27·35	32·55
Calotropis procera	4·66	16·78	21·44
Euphorbia lathyris	6·61	19·13	25·74
Grindelia squarrosa	12·00	10·52	22·52
Helianthus agrestis	7·38	13·45	20·83
H. annuus	7·09	11·73	18·82
H. paradoxus	3·46	19·54	23·00
H. petiolaris ssp. *petiolaris*	1·86	21·00	22·86
Rhus glabra	6·35	46·20	52·55
Trepocarpus aethusae	7·45	20·08	27·53

from Table 1 exactly how these variables are correlated. Because the sunflowers generally have good hybridization potential,[1] a logical question is whether a high rubber-yielding taxon could be crossed with a high protein-yielding taxon and the two factors be selected in the F_2 progeny. The answer to that question would involve a long, costly breeding program. An interim approximation is to examine the intercorrelation of these four variables. If, for example, rubber yield and protein yield have a large negative correlation, this might indicate a metabolic competition for photosynthetate, etc. On the other hand, little or no correlation between yields would be very encouraging for future breeding studies. A principal component analysis (PCA) was run on the 49 samples using four variables (cyclohexane extractable, rubber, methanol extractable, and crude protein yields). The correlation between cyclohexane extractables and rubber yield was only 0·26 (Table 3). This is in spite of the intrinsic correlation since the rubber is a subset of the cyclohexane extractables. The cyclohexane yield was also positively correlated with protein yield (0·25) and essentially uncorrelated with methanol extract yield (0·06).

TABLE 3
Principal Component Analysis (PCA) of Cyclohexane Extractables, Rubber, Methanol Extractables and Protein for the 49 Samples Analyzed

	Correlation matrix			
	Cyclohexane	*Rubber*	*Methanol*	*Protein*
Cyclohexane	–	0·26	0·06	0·25
Rubber	0·26	–	0·06	−0·21
Methanol	0·06	0·06	–	0·28
Protein	0·25	−0·21	0·28	–

Eigenroots (percent of variation extracted): 1, 35·3%; 2, 30·9%; 3, 22·1%.

Percent variance extracted:

Variable	*Factor*			*Total variance extracted*
	1	*2*	*3*	
Cyclohexane	39·18	25·18	24·11	88·47
Rubber	0·97	79·29	6·98	87·24
Methanol	41·64	1·51	51·82	94·97
Protein	59·29	17·54	5·66	82·49

The rubber had a small correlation with the cyclohexane extract yield, essentially none with the methanol extract yield (0·06) and a small negative correlation with protein yield. The methanol extract had a small correlation with the protein yield (0·28, Table 3). The statistical independence of the four variables is further shown by the eigenroots from the PCA. Notice that each of the eigenroots account for roughly equal portions of the variance among the samples (Table 3). This implies little substructure among the variables and this is further evident (bottom, Table 3) in that only rubber has a large loading on any factor (2). We can therefore deduce that, in this sample set, there are not large correlations among any of these four variables. Selection and breeding to maximize more than one of these variables appears to be promising. However, additional samples will be needed from the population level to address this question.

In summary, several sunflower species appear to offer an opportunity for the development of a whole-plant utilization crop[39] based on multiple products[40-42] such as hydrocarbons, rubber, speciality carbohydrates and livestock feed.

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