Seed Oil Characteristics of Wild Field Pennycress (*Thlaspi arvense* L.) Populations and USDA Accessions

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ABSTRACT

The common winter annual weed *Thlapsi arvense* L. (field pennycress) has been identified as a potential new source for fuel and industrial products. Field pennycress offers a very short life cycle allowing opportunities for double cropping in the Midwest region. The objective of this study was to collect and evaluate diverse wild populations of field pennycress for potential inclusion in plant breeding programs.

In the springs of 2009-2013, 80 wild populations of field pennycress seed and corresponding GPS data were collected throughout the central United States. An additional 33 accessions from the North Central Regional Plant Introduction Station in Ames, IA were also evaluated. All samples were analyzed for total seed oil on a dry weight basis, oleic acid (c18:1), linolenic acid (c18:3), erucic acid (c22), and 1000 seed weight at 9% moisture. Total oil content was determined by nondestructive pulsed NMR on whole pennycress seed, and fatty acid methyl esters were quantified utilizing gas chromatography.

Total oil content ranged from 24.43% to 38.71% across the populations collected in the central US with the highest level being seen in a population from Hope, Michigan. A similar range was seen for the USDA accessions of 24.73% to 38.73% with the highest levels found in Alberta, Canada. Oleic acid ranged from 7.72% to 17.13%, linolenic acid levels ranged from 8.39% to 15.25%, and erucic acid levels ranged from 27.46% to 38.41%. For the wild populations: latitude, longitude, and altitude data were not strong predictors for total seed oil content, oleic acid, and 1000sw. However, linolenic levels are correlated with longitude and latitude, while erucic levels are only correlated with latitude. Thousand seed weights ranged from 0.43 g to 1.34g and were not correlated to any GPS data.

Pennycress's unique short growing season, high levels of total oil, and erucic acid content increases its viability for commercialization and potential for plant breeding programs.

Figure 1. Road side collection site of wild pennycress population in Illinois.

INTRODUCTION TO PENNYCRESS

Development of diverse and renewable feed stocks for fuel production is becoming a high priority for the U.S. agriculture sector. However, displacement of crop production that presently supplies much of the world's food from arable land is a concern. One approach to alleviate the dual needs for food and fuel is to rotate both types of crops on the same land in a single extended growing season. The potential new crop, field pennycress (*Thlaspi arvense* L.), grows over the winter and spring months with harvest in late May to early June and will allow full season soybean production on the same acreage immediately following harvest (Phippen and Phippen, 2012). Pennycress can be used as a bio-diesel fuel or as an additive in many industrial based products (Moser et al., 2009).

Pennycress is a member of the mustard family (*Brassicaceae*) and grows throughout the Midwest as a winter or summer annual. The central stem and upper side stems terminate in erect racemes of small white flowers. Flowers are self-pollinated and produce a penny sized heart-shaped, flat seed pod with up to 14 seeds. Each dark brown seed is oval-shaped and is slightly larger than camelina seed (*Camelina sativa*) (Figure 2). Pennycress habitats include: cropland, fallow fields, areas along roadsides and railroads, gardens plots, weedy meadows, and waste areas. This plant prefers disturbed areas, and its capacity to invade higher quality natural habitats is low (Best and McIntyre, 1975).

Figure 2. Pennycress seed pods and seed size comparison to other oilseed crops.

MATERIALS AND METHODS

Population Collections

Wild populations were collected from May to June in 2009-2013. Populations were collected on public land or with permission from land owners. Latitude, longitude, and altitude were recorded for each location using a Garmin Oregon 450T hand held GPS unit. Photographs and an average of 50-500g of seed were taken at each collection site. USDA accessions were grown and maintained at the North Region Central Plant Introduction Station in Ames, Iowa.

Oil and data analysis

Total oil content was determined by nondestructive pulsed NMR (Bruker Minispec PC 120, 180-mm absolute probehead) on whole pennycress seed. Medium-chain triglycerides were extracted and derivatized into fatty acid methyl esters for gas chromatography analysis. The fatty acid methyl esters were analyzed using an Agilent 6890 gas chromatograph with a flame ionization detector. Data was analyzed using analysis of variance with statistical differences declared at P<0.05.

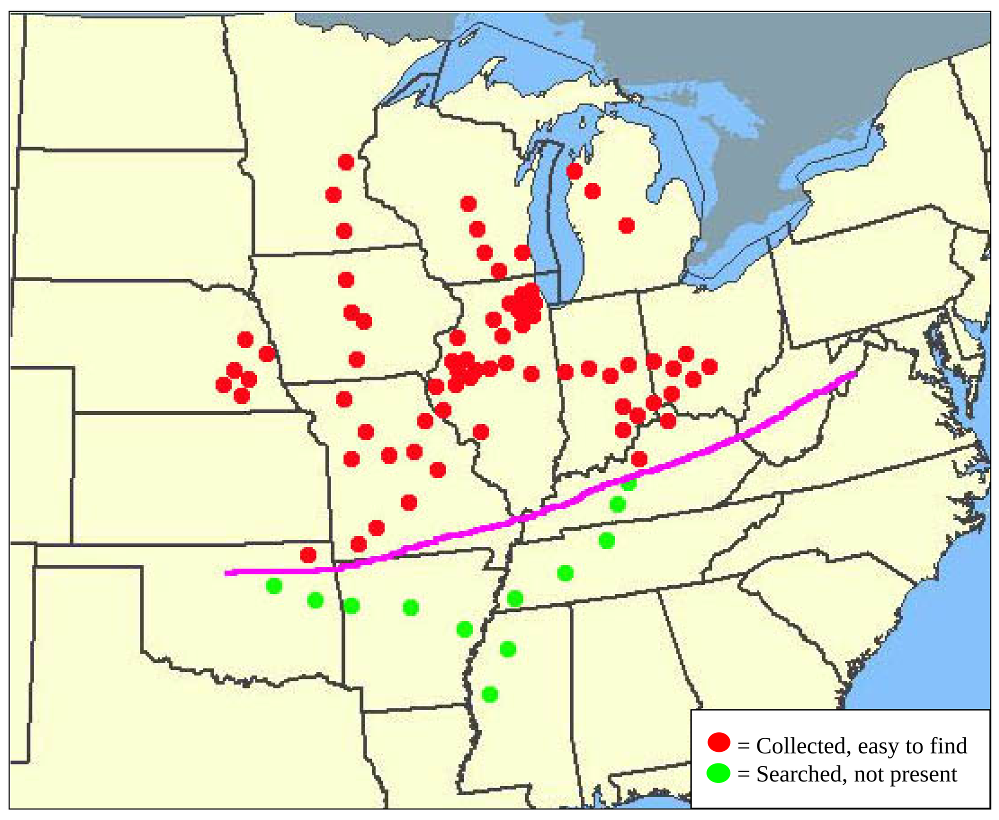

Figure 3. Locations of wild pennycress population collection sites.

Table 1. Seed characteristics of 80 Wild Populations of *Thlaspi arvense* L. Numbers in red indicate highest levels recorded.

Sample Number	Collection Location	% Oil dwb	% Oleic Acid C18:1	% Linolenic Acid C18:3	% Erucic Acid C22:1	1000 sw (g)
11-1	Carthage, Illinois	30.51	12.37	11.83	32.39	0.79
11-2	Quincy, Illinois	31.26	7.72	11.54	32.82	0.94
11-3	Laddonia, Missouri	28.28	13.69	11.34	32.88	1.00
11-4	Holts Summit, Missouri	29.78	12.57	11.57	32.70	0.78
11-5	California, Missouri	26.81	11.78	11.30	32.23	1.00
11-6	Sedalia, Missouri	28.71	11.21	12.00	32.10	0.84
11-7	Carrollton, Missouri	27.45	15.46	10.19	30.21	0.89
11-8	Lincoln, Missouri	32.32	12.55	11.57	32.98	0.98
11-9	Lucas, Iowa	28.69	13.00	9.44	34.44	0.82
11-10	Ankeny, Iowa	27.35	12.00	10.08	37.02	0.88
11-11	Dows, Iowa	28.41	**17.13**	8.37	33.10	0.68
11-12	Glenville, Minnesota	25.14	13.51	9.66	34.83	0.65
11-13	Northfield, Minnesota	31.32	11.75	9.40	35.99	0.86
11-14	Harris, Minnesota	34.04	10.10	10.89	37.59	0.94
11-16	Oxford, Wisconsin	29.55	9.33	14.03	34.19	0.84
11-17	Vienna, Wisconsin	33.79	13.67	10.30	34.55	0.96
11-18	Janesville, Wisconsin	29.50	14.06	10.32	33.67	1.02
11-19	Rochelle, Illinois	29.75	12.15	10.28	36.57	1.14
11-20	Lydon, Illinois	28.90	11.50	10.15	35.31	0.94
11-21	Alexis, Illinois	26.93	12.51	11.82	32.76	1.24
11-22	Macomb, Illinois	30.13	13.38	10.27	31.53	0.90
11-23	Lincoln, Nebraska	24.47	15.05	9.32	30.52	0.67
11-24	Harvard, Nebraska	30.25	8.41	12.15	**38.41**	0.97
11-25	Mead, Nebraska	31.43	9.42	12.95	33.08	0.87
11-26	Lincoln, Nebraska	32.14	9.68	13.11	32.31	0.89
11-27	Omaha, Nebraska	27.10	11.07	10.68	35.38	0.79
11-28	Lincoln, Nebraska	30.37	10.48	12.48	34.13	0.98
Patton 1	Hanna City, Illinois	31.90	11.30	11.30	35.20	0.8
Beecher 2	Hanna City, Illinois	35.00	9.70	13.80	36.90	1.15
W1	McDonough County, Illinois	32.93	9.95	15.04	32.16	1.06
W2	McDonough County, Illinois	26.85	8.38	15.18	35.28	1.03
W3	McDonough County, Illinois	32.89	9.39	15.05	33.46	1.00
W4	McDonough County, Illinois	28.19	11.12	14.98	30.33	1.02
W5	McDonough County, Illinois	33.54	11.38	14.34	34.18	1.14
W6	McDonough County, Illinois	29.82	12.75	14.60	31.18	1.09
W7	McDonough County, Illinois	31.39	10.52	15.08	33.01	**1.34**
W8	McDonough County, Illinois	33.05	11.08	14.48	32.78	1.10
W9	McDonough County, Illinois	34.13	10.78	14.79	34.62	1.25
W10	McDonough County, Illinois	30.88	11.44	14.26	34.46	1.14
W11	McDonough County, Illinois	29.52	8.92	14.68	36.50	1.15
W12	McDonough County, Illinois	29.96	10.88	14.22	34.06	0.97
W13	McDonough County, Illinois	30.13	10.56	14.57	33.78	1.07
13-1	Roxana, Illinois	33.97	10.54	13.66	34.29	0.95
13-2	St. Clair, Missouri	31.70	12.40	13.68	31.43	0.94
13-3	Doolittle, Missouri	32.50	10.57	14.77	32.76	0.91
13-4	Phillipsburg, Missouri	32.67	10.60	14.00	32.30	0.85
13-5	Strafford , Missouri	31.15	12.70	14.33	28.49	0.94
13-6	Afton, Oklahoma	26.65	10.16	13.22	34.94	0.75
13-7	Hillview, Kentucky	30.81	10.38	14.03	33.55	0.96
13-8	Hanover, Indiana	35.00	11.83	14.37	29.78	0.90
13-9	Rexville, Indiana	33.16	10.45	14.20	31.43	0.84
13-10	Versailles, Indiana	33.16	11.86	13.72	31.84	0.79
13-11	Lawrenceburg, Indiana	31.51	11.60	15.07	30.02	0.84
13-12	Petersburg, Kentucky	28.81	8.59	**15.25**	35.36	0.84
13-13	Cleves, Ohio	32.04	11.57	14.08	31.54	0.86
13-14	Lebannon, Ohio	35.41	13.07	14.20	29.09	0.96
13-15	Wilmington, Ohio	31.30	13.75	14.93	27.46	0.96
13-16	Spring Valley, Ohio	26.23	9.87	12.71	33.79	0.70
13-17	Bath, Ohio	30.48	10.42	13.55	34.01	0.87
13-18	Lewisburg, Ohio	27.42	8.39	13.16	35.13	0.72
13-19	Newcastle, Indiana	32.21	12.74	12.42	29.91	0.83
13-20	Greenfield, Indiana	33.81	10.56	14.19	33.65	0.86
13-21	Lizton, Indiana	33.46	13.72	13.49	27.70	0.85
13-22	Covington, Indiana	34.82	10.10	14.09	33.91	0.83
13-23	Oakwood, Illinois	27.24	13.72	11.12	32.34	0.72
13-24	LeRoy, Illinois	28.44	8.79	14.92	36.58	0.81
13-25	San Jose, Illinois	27.71	11.20	13.11	37.28	0.85
13-26	Lewiston, Illinois	27.39	9.24	11.87	35.07	0.71
13-27	Adair, Illinois	24.43	11.87	13.48	31.02	0.57
13-28	Naperville, Illinois	34.74	9.19	13.86	35.24	0.86
13-29	Eola, Illinois	36.01	8.76	14.67	37.35	0.86
13-30	Aurora, Illinois	15.95	10.42	13.14	29.28	0.43
13-31	Metea Valley, Illinois	13.52	9.51	14.02	28.83	0.56
13-32	Oak Brook, Illinois	33.56	10.47	13.50	34.93	1.04
13-33	Batavia, Illinois	34.22	9.23	13.92	35.87	0.82
13-34	Geneva, Illinois	31.04	8.47	15.06	36.00	0.84
13-35	Hope, Michigan	**38.71**	9.27	13.10	35.80	1.01
13-36	Cadillac, Michigan	31.74	10.75	13.57	34.08	0.95
13-37	Frankfort, Michigan	32.00	9.60	13.83	34.67	1.01
13-38	Pleasant Prairie, Wisconsin	28.37	9.67	13.06	35.83	0.82
13-39	Rolling Meadows, Illinois	32.45	10.11	13.34	36.74	1.15
13-40	Macomb, Illinois	30.99	10.61	13.42	36.12	0.97
Total Range		13.52-38.71	7.72-17.13	8.37-15.25	27.46-38.41	0.43-1.34

Table 2. Oil % on a dry weight basis, oil constituents, and 1000 seed weight correlated to GPS location for 80 Wild Populations of *Thlaspi arvense* L. Numbers in red are significant at 0.05 probability level.

	R square				
	% Oil dwb	Oleic Acid C18:1 D9	Linolenic Acid C18:3	Erucic Acid C22:1	1000 sw (g)
Latitude	0.000	0.004	0.105	**0.185**	0.001
Longitude	0.042	0.020	**0.186**	0.029	0.002
Lat + Long	0.042	0.024	**0.291**	**0.214**	0.003
Altitude	0.026	0.026	0.072	0.013	0.066

Table 3. Seed characteristics of 34 USDA accessions of *Thlaspi arvense* L. Numbers in red indicate highest levels recorded.

Accession Number	Origin	% Oil dwb	% Oleic Acid C18:1	% Linolenic Acid C18:3	% Erucic Acid C22:1	1000 sw (g)
Ames 22461	Poland	32.33	11.65	11.77	33.80	1.24
Ames 23761	Ontario, Canada	32.70	8.48	13.16	37.42	0.92
Ames 24499	Former Serbia	31.04	11.35	11.37	36.90	0.81
Ames 29118	Illinois, USA	27.64	9.17	13.45	37.14	0.79
Ames 29509	Ohio, USA	36.92	10.80	12.47	35.71	1.00
Ames 29512	Ohio, USA	27.30	8.64	12.46	37.94	0.60
Ames 29513	Iowa, USA	30.62	9.47	13.38	34.61	0.84
Ames 30982	Iowa, USA	32.44	9.53	14.26	34.89	0.83
Ames 30983	Illinois, USA	32.05	10.35	12.70	34.26	0.87
Ames 30984	South Dakota, USA	36.08	9.61	14.13	33.87	0.93
Ames 30985	South Dakota, USA	30.96	11.97	13.01	33.37	1.01
Ames 30997	Colorado, USA	24.73	**12.65**	12.14	29.81	0.76
Ames 30999	Colorado, USA	33.39	11.09	14.09	32.45	0.90
Ames 31012	Colorado, USA	34.04	9.13	14.68	32.78	0.81
Ames 31018-L1	Colorado, USA	33.96	11.36	13.83	32.52	0.92
Ames 31018-L2	Colorado, USA	32.05	11.30	13.02	35.20	**1.33**
Ames 31021	Colorado, USA	36.01	8.66	**14.98**	36.81	0.91
Ames 31023	Colorado, USA	34.46	9.28	13.99	36.88	0.96
Ames 31026	Colorado, USA	27.74	10.63	13.38	31.34	0.81
Ames 31487	Ontario, Canada	33.80	10.07	13.03	37.88	0.86
Ames 31488	Ontario, Canada	35.21	11.22	12.65	35.15	1.00
Ames 31489	Saskatchewan, Canada	36.09	11.32	13.12	34.74	1.04
Ames 31490	Saskatchewan, Canada	32.15	8.78	13.47	37.23	1.04
Ames 31491	Saskatchewan, Canada	31.26	8.70	12.73	36.45	0.92
Ames 31492	Saskatchewan, Canada	36.00	7.63	14.21	**38.95**	0.97
Ames 31493	Saskatchewan, Canada	34.54	9.49	12.98	36.97	1.18
Ames 31500	Alberta, Canada	**38.73**	8.29	13.12	37.68	1.14
Ames 31501	Manitoba, Canada	37.09	10.11	12.24	36.90	1.04
PI 633414	Thuringia, Germany	27.03	9.88	12.90	35.79	0.81
PI 633415	Saxony, Germany	25.86	9.22	10.33	36.52	0.72
PI 650284	Thuringia, Germany	31.56	10.03	8.90	35.86	0.84
PI 650285	Saxony, Germany	30.22	10.96	11.64	35.48	0.94
PI 650286	Saxony, Germany	26.88	10.65	9.09	35.96	0.81
PI 650287	Bas-Rhin, France	29.12	8.73	12.10	38.11	0.89
Total Range		24.73-38.73	7.63-12.65	8.90-14.98	29.81-38.95	0.60-1.33

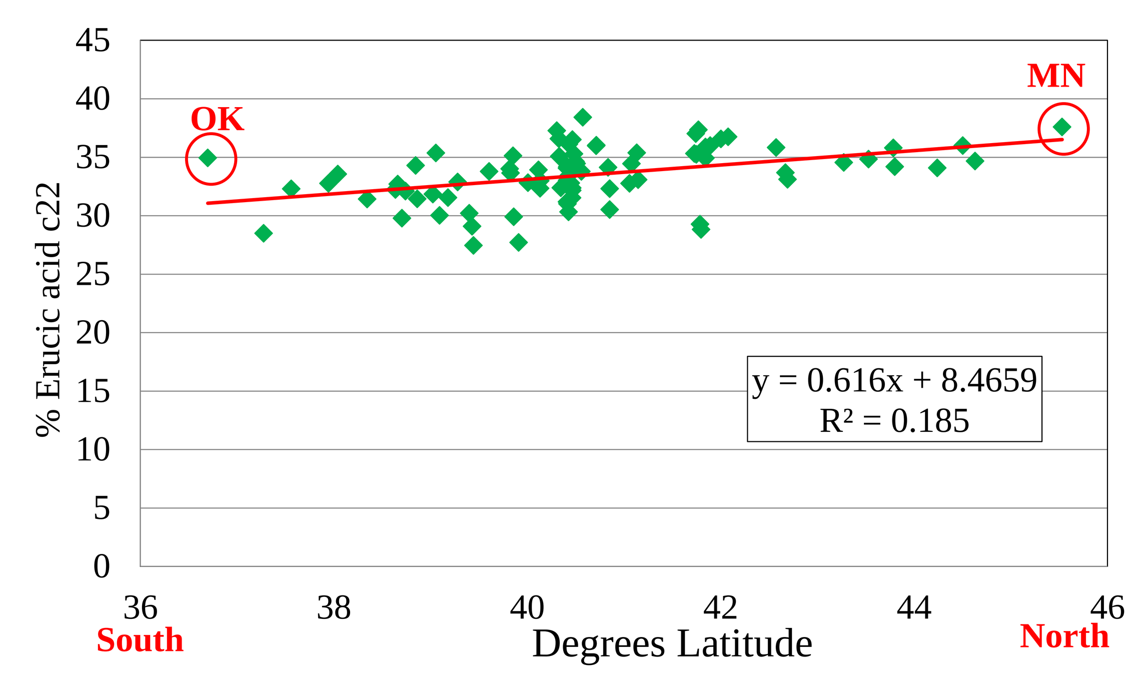

Figure 4. Regression analysis of percent erucic acid and latitude of collection site.

RESULTS

Our collection effort has resulted in 80 new wild populations of field pennycress. All populations were analyzed for total seed oil content, oil constituents, and 1000 seed weight based on their original collection locations (Table 1). Total percent seed oil ranged from 13.52-38.71 across all populations. Oleic acid, linolenic acid, and erucic acid ranged from 7.72-17.13%, 8.37-15.25%, and 27.46-38.41%, respectively. One thousand seed weight ranged from 0.43-1.34g. The low seed weights of 13-30 and 13-31 were probably due to collection of immature seed. The resulting seed characteristics correlated to GPS coordinates and altitude of the collection sites can be seen in Table 2. Only linolenic acid was significantly effected by longitude. Erucic acid was effected by latitude (Figure 4). When considering both latitude and longitude, linolenic acid and erucic acid content were significant (Table 2). In addition, 34 of the currently available USDA accessions were analyzed and displayed narrower ranges for seed and seed oil characteristics (Table 3). Although accessions were from diverse areas, the seed used in the analysis were from seed increases grown in Ames, Iowa.

CONCLUSIONS

The pennycress populations and accessions identified with high seed oil content, high levels of fatty acid methyl esters, and larger seed sizes can now be integrated into current breeding programs to further the development of pennycress as a viable new crop for US agriculture. Pennycress breeding is currently focused on improving the agronomic traits of flowering time, seed dormancy, seed size, and overall plant vigor. Commercial efforts to advance pennycress reached a level of ~500 acres in 2013. The oils from pennycress seed can be easily transformed into fuels and chemicals that are highly compatible with existing petroleum analogs.

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