

**Geographic variation in natural rubber yields in natural populations of
Helianthus annuus (Asteraceae, Sunflowers)**

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ABSTRACT

Populations of *Helianthus annuus*, ranging from eastern Oklahoma to coastal southern California, were sampled and the yields of natural rubber (NR) from leaves determined by Accelerated Solvent Extraction (ASE). The highest yielding populations were Mill Creek, UT with a yield of 20.8 mg/ g and in the Waco, TX area with 14-18.2 mg/ g leaf DW. The lowest yields were in Preston, ID (1 mg/g), Redmond, OR (2 mg /g), Eagle Lake, NM (2.7 g/ mg), Post, TX (3 mg/g) and Quanah, TX (3 mg/ g). The highest yielding individuals were found in Mill Creek, UT (42 mg /g) and McLennan Co., Waco, TX (31 mg/ g). The appears to be no geographical relationship to the rubber yields. The highest yielding populations are in very different eco-systems with different soils, climates and ecologies. Adjacent populations are often quite different in the concentration of rubber. The most variable populations were San Diego, CA (0 - 16 mg/ g), Logan, UT - Preston, ID (0 - 12 mg/g), Dodge City, KS (0, 0, 7,8,10 mg/ g), and Meade, KS (2, 2, 4, 18 mg/ g). There is some geographic trends in rubber variation in central Kansas. But, other highly variable populations are isolated in different ecosystems. Additional studies needed to more fully understand the variation. Published on-line www.phytologia.org *Phytologia* 100(1): 62-70 (Mar 16, 2018). ISSN 030319430.

KEY WORDS: *Helianthus annuus*, Sunflower, natural rubber, geographic variation.

Stipanovic et al. (1980) reported on rubber content from 23 wild *Helianthus* species, 2 commercial varieties and one wild hybrid, grown at the USDA Bushland, TX garden. The rubber yields from these taxa varied from 16.7 mg /g (DW leaves) in *H. agrestis* to 1 mg/ g in *H. neglectus*.

Adams and Seiler (1984) expanded the Stipanovic et al. (1980) study by examining 39 taxa of sunflowers, grown in the garden at USDA Bushland, TX), for their rubber, cyclohexane (hydrocarbon), methanol (resins) and protein concentrations. They reported that rubber varied from 17.8 mg/ g (*H. californicus*, Napa, CA and *H. resinosus*, Collins, MS to 1 mg/ g (*H. neglectus*, Kermit, TX). Seiler, Carr and Bagby (1991) analyzed 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. 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.

Recently, a new method was developed for the solvent extraction of rubber from *H. annuus* (Pearson et al. 2010a) using an Accelerated Solvent Extraction (ASE) machine. Yields of natural rubber for *H. annuus* leaves ranged from 9 mg/ g to 17 mg/ g rubber in sunflower cultivars grown under field conditions (see Fig. 4, Pearson et al. 2010b). ASE was found to be of considerable use in quickly screening numerous plants for rubber (and resins, etc.). There appear to be no publications concerning the geographical variation in the yields of rubber from *H. annuus*. ASE technology provided us an opportunity to screen large numbers of plants for rubber content.

Adams, et al. (2017a) screened natural populations of *H. annuus* for pentane extractable hydrocarbons and found the highest yielding populations in the Texas panhandle, where the plants were stressed by wind, insect and disease damage (Fig. 1). Plants from these high yielding populations returned to typical (lower) yields when grown under lush conditions in a greenhouse (Adams, et al. 2017b).

The lowest yields of hydrocarbons were found in AZ and NM and throughout the southwest (Fig. 1). Notice (Fig. 1) the WO (Woodward, OK) population had a very low yield (2.6%). The leaves were glabrous, suggesting the WO site is of hybrid origin from locally cultivated sunflowers.

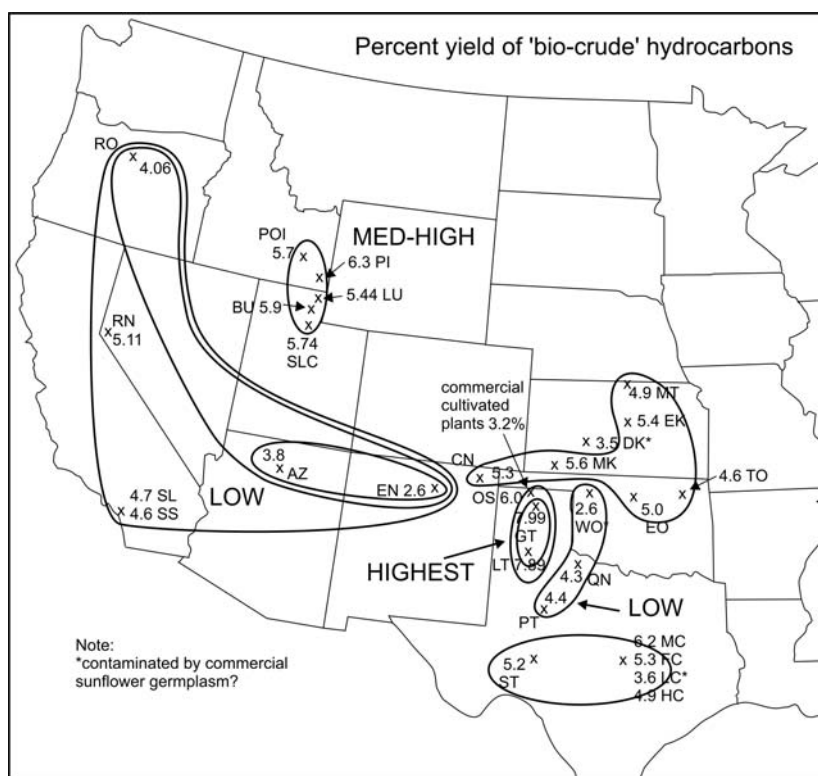


Figure 1. Geographic variation in % yields of HC by population. The asterisk (*) at the WO population indicates that the population is likely of hybrid origin between native and cultivated sunflowers. Note the low yield (3.2%) from commercial sunflowers near Oslo, TX. See text for discussion.

Our large collection of air-died leaves of *H. annuus* was found to be suitable for ASE extraction of rubber. The purpose of this report is to present new information on geographic variation of the yields of rubber in native, annual sunflower, *H. annuus*. This is a continuation of our research on sunflowers (Adams and TeBeest, 2016; Adams, et al. 2016).

MATERIALS AND METHODS

Population locations - see Appendix I.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). Leaf rubber concentration was quantified by Accelerated Solvent Extraction (ASE) using a Dionex ASE 200 (Thermo Fisher Scientific, Waltham, MA). Dried and ground samples were weighed to 0.5000g ± 0.0100g and mixed with approximately 14g of inert Ottawa sand (20-30 mesh) (Thermo Fisher Scientific, Waltham, MA) before loading into 11mL stainless steel extraction cells. The sequential three-solvent extraction protocol (Pearson et al., 2010a) started with two acetone extractions (16 minutes each) at 40°C, then five methanol extractions (5 minutes each) at 60°C, and finally two hexane extractions (16 minutes each) at 40°C. Collection vials containing the mixture of extracted analyte and solvent were vortexed and then poured into pre-weighed 60ml aluminum pans (Fisherbrand, Fisher Scientific). Pans were placed in a fume hood at ambient temperature for 24 hours to evaporate the solvent. Once completely dry, pans were reweighed and analyte concentration was calculated.

RESULTS

The yields of rubber by population are given in Table 1 and shown in Figure 2. The highest rubber yielding populations (red, Fig. 2) were MCUT (20.8 mg/g, Mill Creek, UT), in the Waco, TX area (14-18.2 mg/ g), and POCID (12 mg/g, Pocatello, ID). Several low yielding populations are shown in blue (Fig. 2): Redlands, OR, Preston, ID- Logan-UT, Capulin, NM - Eagle Nest, NM - Oslo, TX, Dodge City - Ellsworth, KS and Post - Quanah, TX.

It might be noted that the lowest elevation population (except San Diego) was Waco, TX and the highest elevation was MCUT (Salt Lake City, UT). Both of the areas were high in rubber concentration.

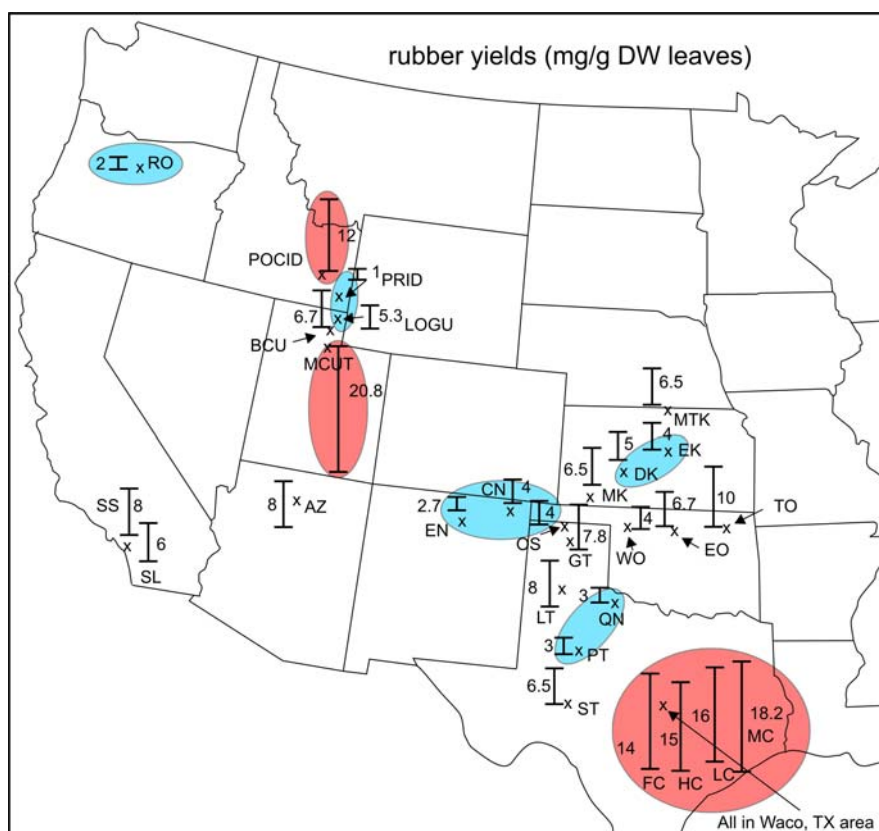


Figure 2. Geographic variation in yields of rubber (mg/ g DW leaves) from natural populations of *H. annuus*. Bar values are the population average. High yielding populations are in red, and low yielding are in blue.

The populations around Waco, TX (FC, HC, LC, MC) are rather uniform in their averages. The variation in northern UT and southern ID, is a mosaic with 2 low yielding populations (PRID, LOGU) flanked by 2 high yielding populations (MCUT, POCID).

To examine intra-population variation, rubber yields for individual plants were mapped by population (Fig. 3). The highest yielding plant (42 mg/ g) was from the MCUT population (green, Fig. 3). Yields from plants in the MCUT population ranged from 10 to 42 mg/ g. The second highest yielding plant in this study (31 mg/ g) was from McLennan County, Texas. In the Waco, TX area (green, Fig. 3), yields ranged from 12 to 31 mg/ g. Large numbers of plants were analyzed from GT (Gruver, TX) and LT (Lake Tanglewood, TX), so frequency distribution graphs (yellow) were constructed. The GT population had a mode of 8 mg/ g and then tailed to 16 mg/ g (Fig. 3). The LT population appeared to be bi-modal with 3 plants with 2 mg/ g, 1 with 4 mg/ g, 1 with 6 mg/ g and then most with 8 - 14 mg/ g (Fig. 3).

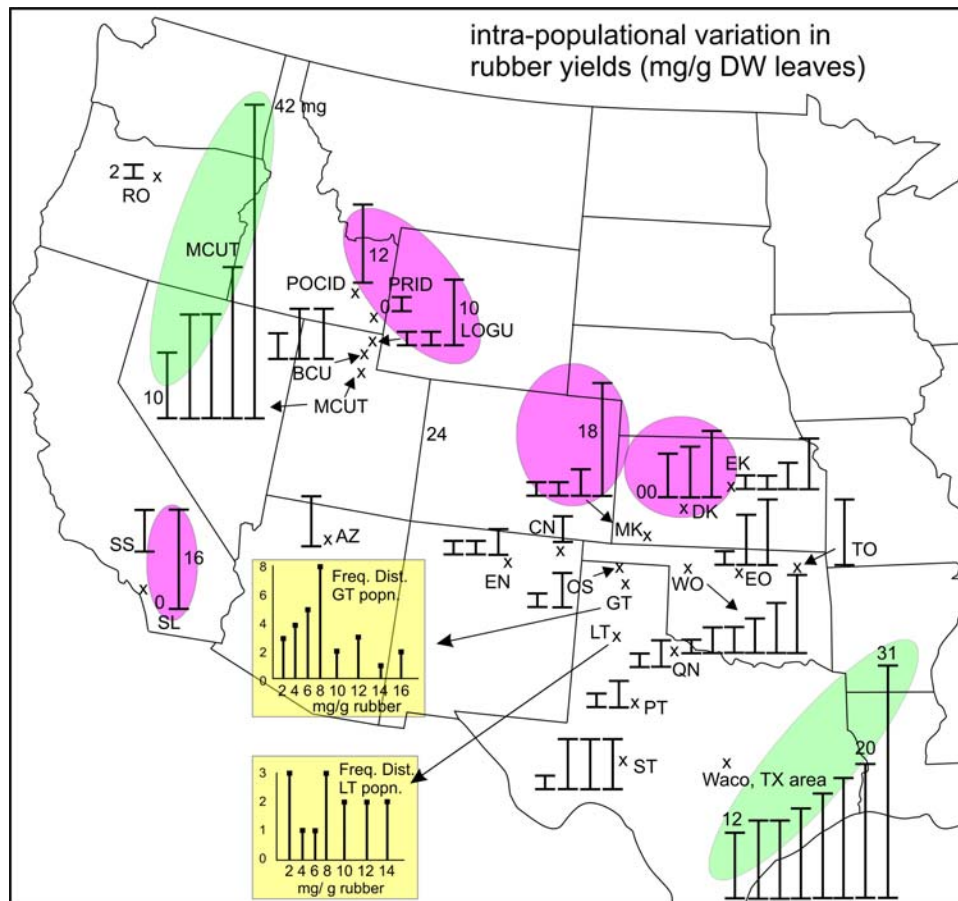


Figure 3. Intra-population variation in rubber yields (mg/ g DW leaves). Note that populations GT and LT contained too many samples to show individually, so they as summarized in Frequency Distribution graphs (yellow). The high yielding individuals at MCUT (Mill Creek, UT) and in the Waco, TX area are shaded in green. Populations with polymorphic yields are shaded in magenta.

Several populations appeared to have a polymorphic (or chemotypes, low and high yields) production of rubber. In general, these populations (magenta, Fig. 3) contained individuals with no (zero detected) rubber, and plants with some or considerable amounts of rubber. For example, the DK (Dodge City, KS) population had 2 plants with 0 (none) and 3 plants with 7, 8, 10 mg/ g yields of rubber. The Meade, KS (MK) population had 2, 2, 4 mg/ g plants and one plant with 18 mg/ g yield. Likewise,

northern Utah - southern Idaho plants contained 0, 2, 2, 4, 10, 12 mg/ g which is suggestive of low- and high- rubber chemotypes. Additional research will be needed to fully ascertain if these chemotypes exist, or if the range of variation is continuous when additional samples are analyzed. Only three samples were analyzed from San Diego, CA (SL, SS), and they yielded 0, 6, and 18 mg/ g.

Plants that appear to have no rubber could provide useful parents when crossed with high yielding plants to examine the genetic basis of rubber yields in sunflowers (research in progress).

It is interesting to examine the distribution of HC yields (Fig. 1) vs. rubber yields (Fig. 2). Notice that the highest yielding HC populations (GT, LT, Fig. 1) are quite intermediate in their rubber yields (Fig. 2). Likewise, the high yielding rubber populations (FC, HC, LC, MC, Fig. 2) are just medium yielding HC populations (Fig. 1). However, the low HC yielding populations (AZ, EN, RO, PT, QN, WO, Fig. 1) are also low in rubber (Fig. 2). Because we have shown (Adams, et al. 2017b) that high yields of HC are induced by stress factors (insect, wind and disease conditions), it is not surprising that the high HC yielding populations do not necessarily correlate with high rubber yielding populations. In fact, the correlation between HC yields and rubber yields was found to be $r=0.195$. Thus, only 3.8% of the variance in HC is explained by variation in rubber yields. Although the hydrocarbon fraction (HC) in *H. annuus* contain considerable amounts of terpenoids (Adams et al. 2017c; Gershenzon et al. 1981), the induction of mono-, sesqui-, di- and tri-terpenoids by stresses does not seem correlated the amount of rubber (a terpenoid polymer).

In summary, considerable geographic variation was found in the yields of rubber in native sunflowers (*H. annuus*) that varied from 0 (zero) to 42 mg/ g. At present (until plants are grown in a garden/ greenhouse), the role of genetics vs. environment is not known in sunflower rubber production. Nor is the range of geographic variation fully known for *H. annuus*. New collections in the western portion of the range of *H. annuus* are being analyzed and this will more fully complete the knowledge of geographic variation in the yields of rubber in natural populations.

ACKNOWLEDGEMENTS

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Table 1. Yields of ASE natural rubber (mg/g leaves DW) from natural sunflower (*H. annuus*) populations. Yields are rounded to nearest mg. Specimen collection numbers are of *Robert P. Adams* collection series.

popn id		ASE rubber Avg.	ASE rubber, individuals
AZ	15021 Camp Verde, AZ (10), 8/27/16 Thornburg	8.06	8
BCU	15022, Brigham City, UT, (10), 9/2/16	6.67	4,8,8
CN	14981 Capulin, NM (10), TeBeest ns	4	4
DK	14940 H. ann. (10), 7/7/16, Dodge City, KS	5.0	0,0,7,8,10
EK	14941 H. ann. (10), 7/7/16, Ellsworth, KS	4.0	2,2,4,8
EN	14980 Eagle Nest, NM,(10), TeBeest ns	2.67	2,2,4
EO	14943 H. ann. (10), 7/9/16, Enid, OK EOK (10)	6.67	2,8,10
FC	14977 Falls Co. Satin, (10), Holmes 16656	14	14
GT1	14952 H. ann., Gruver, TX, GT1 1-10, mi south 7/16/19	6.9	2,4,4,4,8,10,16
GT2	GT2 11-20, 1mi south 7/29/16	8.6	2,6,6,8,8,14,16
GT3	GT 21-30, 2 mi E, Rodeo Grounds 8/1/16	9.7	8,8,8,8,12,12,12
GT4	GT 31-40, 1 mi south 8/15/16	6.0	2,4,6,6,6,8,10
HC	14979 Hill Co., (10), Holmes 16661	15.0	12,18
LC	14978 Limestone Co. Mt. Calm, (10), Holmes 16658	16	16
LOGU	15023, Logan, UT, (10), 9/2/16	5.3	2,4,10
LT1	14947 H. annuus, Lake Tanglewood, TX LT (10), 7/12/16	7.7	2,6,8,8,8,10,12
LT2	Lake Tanglewood, LT2, (10), 2nd collection 7-20-16	8.3	2,2,4,10,12,14,14
MC	14976 McLennan Co. (10), Holmes 16654 1-10	18.2	10,12,18,20,31
MCUT	15026, Mill Creek, Salt Lake City, UT (10), 9/4/2016	20.8	10,14,14,24,42
MK	14939 H. annuus (10), 7/7/16, Meade, KS,	6.5	2,2,4,18
OS	14946 H. annuus (10), 7/12/16, Olso, TX native in grass	4	2,6
POCID	15025, Pocatello, ID, (10), 9/2/16	12	12
PRID	15024, Preston, UT, (10), 9/2/16	1.0	0,2
PSTX	14935 H. ann. (10), 6/4/16, Post, TX PST (10)	3	0,2,4
QN	14936 H. ann. (10), 6/5/16, Quanah, TX 6/5/16 QT (10)	3.0	2,4
ROR	15027, Redmond, OR, (10), 8/30/16	2	2
SL	14951 H. ann. San Diego, CA large lvs, (10), 7/6/16, Bartel SD8-1...10	8	0,16
SS	14950 H. ann. San Diego, CA small lvs, (10), 7/6/16, Bartel SD6-1...10	6	6
ST	14945 H. ann. (10), 7/12/16, (11) ex Sonora, TX (cult Oslo, TX) ex PI413168	6.5	2,8,8,8
TO	14942 H. ann. (10), 7/9/16, Tulsa, OK	10	10
WO	14944 H. ann. (10), 7/9/16, Woodward, OK smooth lvs, hybrid with cultivated sunflowers.	4.3	2,2,4,4,6,8

Appendix I Population locations.*Helianthus annuus L. below:*

common along railroad and roadside in sandy soil. flowering. 5.3 mi SE of Post TX on US 84, 33° 01' 53" N, 101° 11' 25" W, 2300 ft, Date: 4 June 2016 County: Garza; State: TX

Coll. Robert P. Adams No. 14935

common along fence row and roadside in sandy soil. flowering. 7 mi SE of Quanah, TX on US 287, 34° 15' 57" N, 99° 36' 46" W, 1450 ft, Date: 5 June 2016 County: Hardeman; State: TX

Coll. Robert P. Adams No. 14936

1.5 mi s of Meade, on KS23, low area in edge of wheat field, 100s of plants in population, but generally uncommon. ~5% flowering. 37° 15' 49" N, 100° 20' 40" W, 2433 ft, Date: 7 July 2016; County: Meade; State: KS

Coll. Robert P. Adams No. 14939

8.5 mi NE of Dodge City, US 50, several on dirt piles of highway dept., but generally uncommon. ~5% flowering, 37° 47' 06" N, 99° 53' 14" W. 2534 ft. Date: 7 July 2016, County: Ford; State: KS

Coll. Robert P. Adams No. 14940

1.6 mi e of Ellsworth on KS140, on fence row on s side of wheat field, 20 plants, but generally uncommon. ~10% flowering. 38° 44' 24" N, 98° 11' 53" W, 1600 ft, Date: 7 July 2016, County: Ellsworth; State: KS

Coll. Robert P. Adams No. 14941

15 plants on disturbed area next to South Ash St. (just south of OK364), but generally uncommon, Jenks, OK (sw suburb of Tulsa). ~5% flowering. 36° 00' 57.85" N, 95° 58' 07.61" W, 613 ft, Date: 9 July 2016, County: Tulsa; State: OK

Coll. Robert P. Adams No. 14942

5.5 mi e of Enid on OK412, on fence row, side of wheat field, few plants but generally uncommon. ca 5% flowering, ~5% flowering. 36° 23' 51" N, 97° 46' 51" W, 1160 ft., Date: 9 July 2016, County: Garfield; State: OK

Coll. Robert P. Adams No. 14943

smooth leaves! 2.8 mi e of Woodward on OK412, on fence row, side of grass field, few plants but generally uncommon. ca 5% flowering mostly pre-flowering. 36° 25' 53" N, 99° 20' 28" W, 1880 ft., Date: 9 July 2016, County: Woodward; State: OK

Coll. Robert P. Adams No. 14944

cultivated at Oslo, TX, from seed (USDA PI413168-NC7) ex Sonora, TX. 80% flowering, 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft, Date: 12 July 2016, County: cult in Hansford; State: TX.

Coll. Robert P. Adams No. 14945

native in grassland, JP & Amy TeBeest farm, 1 mi. s of Oslo Lutheran Church. ~5% flowering. 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft., Date: 12 July 2016, County: Hansford; State: TX

Coll. Robert P. Adams No. 14946

2- 3 ft plants, lots of resin on petioles and leaf veins, many sugar (black) ants, most with wilted leaves, very dry in July, common in native grass and on disturbed roadside, brush dump area, Lake Tanglewood, ~50% flowering, 35° 04' 23.7" N, 101° 47' 29.0" W, 3239 ft., Date: 12 July 2016, County: Randall; State: TX

Coll. Robert P. Adams No. 14947

cultivated at Oslo, TX, from seed (USDA PI 531028) ex Idaho, 80% flowering. 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft, Date: 12 July 2016, County: cult in Hansford; State: TX

Coll. Robert P. Adams No. 14948

plants 2' tall, with small leaves, along San Pasqual Rd, 33° 05' 08.2" N, 117° 01' 46.2" W, 353 ft.
Date: 6 July 2016, County: San Diego; State: CA, Coll. Jim A. Bartel 1636
Lab Acc. Robert P. Adams No. 14950

plants to 8' tall, with large leaves, along San Pasqual Rd, 33° 05' 08.2" N, 117° 01' 46.2" W, 353 ft/ Date: 8 July 2016, County: San Diego; State: CA, Coll. Jim. Bartel 1636
Lab Acc. Robert P. Adams No. 14951

2-3' tall, 10% flowering, lots of damage to leaves by grasshoppers, etc., some with many black (sugar) ants, copious resin at base of leaves, along fence row, on TX 206, 1-5:1.2 mi s, 6-10: 1.3 mi. s of Gruver, TX. 36° 14' 52" N, 101° 24' 52" W, 3161 ft,
Date: 16 July 2016, County: Hansford; State: TX
Coll. Robert P. Adams No. 14952

cultivated, irrigated near Oslo, TX, on Slough farm. at R-5.1 stage. 36° 22' 42.17" N, 101° 37' 21.4" W, 3350 ft., leaves mostly smooth. Date: 17 July 2016, County: cult in Hansford; State: TX
Coll. Robert P. Adams No. 14953

Coll. Walter Holmes

(WCH16654) McLennan Co. 12th Street at Flat Creek, Robinson (Waco), 27 July 2016, Walter Holmes

Lab Acc. Robert P. Adams 14976

(WCH 16656) Falls Co. near Satin on FR 434, prairie roadside, 28 July 2016, Walter Holmes

Lab Acc. Robert P. Adams 14977

(WCH 16658) Limestone Co. near jct of Limestone Co roads 102 and 106, south of Mt. Calm, prairie, 29 July 2016, Walter Holmes

Lab Acc. Robert P. Adams 14978

(WCH 16661) Hill Co. US Hwy 84, West of Mt. Calm near jct with West Somers Lane, 29 July 2016, Walter Holmes

Lab Acc. Robert P. Adams 14979

roadside waste area, Eagle Nest, NM, 36° 33.650' N, 105° 15.969' W, 8260 ft, Date: 8 Aug 2016, County: Colfax; State: New Mexico, Coll. Amy TeBeest

Lab acc. Robert P. Adams 14980

roadside waste area, Capulin (city), NM, some grasshopper damage, 36° 44.527' N, 104° 00.178' W, 6820 ft,

Date: 8 Aug 2016, County: Union; State: New Mexico, Coll. Amy TeBeest

Lab acc. Robert P. Adams 14981

cultivated at Oslo, TX, from seed (USDA PI1413033), ex Montrose, KS. Date: 2 Aug 2016, Coll. Amy TeBeest,

Lab acc. Robert P. Adams 14982

along roadsides. 16-18 mi east of Camp Verde on AZ 260. 34.489° N, 111.597° W, 5900 ft, Date: Aug. 27, 2016, County: Yavapai; State: AZ, Coll. David Thornburg ns,

Lab. acc. Robert P. Adams No. 15021

vacant lot behind new WalMart on disturbed soil. flowering and seeding, multiple branches. W1500S, 775W, Brigham City, UT, 41° 28' 57" N, 112° 01' 40" W, 4250 ft, Date: Sept. 2, 2016, County: Boxelder; State: UT

Coll. Robert P. Adams No. 15022

vacant lot behind new stores on disturbed soil on US 91 and E2000N, flowering and seeding, multiple branches. Logan, UT, 41° 46' 09" N, 111° 49' 59" W, 4506 ft, Date: Sept. 2, 2016, County: Cache; State: Utah

Coll. Robert P. Adams No. 15023

vacant lot in new subdivision on disturbed soil off of OR hwy 34/36 & just on n edge of Preston, flowering and seeding, multiple branches. 42° 06' 40" N, 111° 52' 01" W, 4703 ft, Date: Sept. 2, 2016, County: Franklin; State: Idaho

Coll. Robert P. Adams No. 15024

next to sidewalk, on slope, next to freeway (I15) access south, flowering and seeding, multiple branches, Pocatello, ID. 42° 52' 49" N, 112° 25' 35" W, 4625 ft, Date: Sept. 2, 2016, County: Bannock; State: Idaho
Coll. Robert P. Adams No. 15025

next to sidewalk, flowering and seeding, multiple branches. common along sidewalks, Mill Creek, UT. s side of I80 on 2000 E, east side of 2000E. 42° 52' 49" N, 112° 25' 35" W, 4625 ft, Date: Sept. 3, 2016, County: Salt Lake; State: Utah
Coll. Robert P. Adams No. 15026

disturbed area, vacant on SW Airport Way, ~373m sse of jct SW Airport Way & Veterans Way. Redmond, OR, 44° 15' 30" N, 121° 09' 54" W, 3035 ft, Date: Sept. 3, 2016, County: Redmond; State: Oregon
Coll. Mark R. Corbet, ns, Lab Acc. Robert P. Adams No. 15027

disturbed area, Neil Rd and west frontage road on I580, s of Reno, NV. 39° 28' 11.6" N, 119° 47' 20.4" W, 4485 ft, Date: Sept. 5, 2016, County: Washoe; State: Nevada
Coll. Chauncey Parker, ns, Lab Acc. Robert P. Adams No. 15029.