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Agronomic and natural rubber characteristics of sunflower as a rubber-producing plant

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ABSTRACT

Sunflower (*Helianthus annuus* L., Asteraceae) is a genus native to North America and is a potential natural rubber (NR) producing crop. The objectives of the study were to: (1) evaluate commercial sunflower cultivars to determine biomass production and how they partition biomass into leaves, stems, and head, (2) determine how removing the head affects biomass partitioning, (3) determine latex concentration and yield in commercial sunflower cultivars and a diversity of genetic sunflower material, and (4) characterize several quality factors pertaining to the latex produced by commercial sunflower cultivars and a diversity of genetic sunflower material. Field performance tests were conducted at the Western Colorado Research Center at Fruita, Colorado for three growing seasons (2001, 2002, and 2003). Latex was found almost entirely in the leaves of young and mature sunflowers. No latex was found in mature stems or in the pappus of the flowers. On average, sunflower partitioned biomass into 18% leaves, 38% stems, and 44% heads. With the head removed, sunflower partitioned biomass into 33% leaves and 67% stems. Sunflower cultivars exhibited considerable genetic variation for biomass partitioning and also for NR yield and quality. Sunflower synthesized NR with 95–97% being low molecular weights ranging from 66,000 to 74,000 g/mol and a small, remarkable percentage (~5%) of NR being higher molecular weight (~600,000 g/mol). The potential for increasing latex production in sunflower appears possible, given that current NR levels are low and reasonable advances in NR production in sunflower plants through plant breeding and genetic engineering might be achieved. Based on the genetics of the sunflower cultivars included in our study, it appears that some cultivars would be more responsive to plant breeding for increased leaf mass than others. The development of sunflower cultivars suitable for commercial production of NR will require significant improvements in the quantity and quality of NR produced in the plant.

1. Introduction

Natural rubber (*cis*-1,4-polyisoprene) is an indispensable biomaterial for our modern standard of living. More than $2 billion of NR is imported into the United States each year (Rubber Industry Report, IRSG, 2009) as well as vast quantities of finished goods. The United States is dependent on imports of NR from countries located halfway around the world. This creates a long supply chain which could easily be disrupted by natural disaster or by political or military action from foreign powers.

Almost all NR used in commerce comes from a single plant species, the Brazilian rubber tree (*Hevea brasiliensis* Muell. Arg., Euphorbiaceae). There are at least 2500 other plant species that produce latex (Bowers, 1990). Latex is a complex colloidal suspension comprised of water, rubber, resin, proteins, starches, sugars, oil, tannins, gums, and other substances. Most of these latex-producing species are tropical and produce low quality and small amounts of NR. Furthermore, most are wild and technical information about them is scant. There are other plant species that do produce high quality NR similar to *Hevea* (Buranov et al., 2005), but they are not domesticated and most are not suited to mechanized agriculture. Domesticating wild plant species requires a large scientific effort

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in research and development, is expensive, requires a long-term commitment, and is risky.

Over the years, a number of researchers have investigated the potential of plant species that would be suitable for NR production in the United States. Buchanan et al. (1978) evaluated 100 plant species found in Illinois for their potential as hydrocarbon and NR-producing crops. They concluded, "It appears technically and economically feasible to develop a U.S. crop that is as productive in hydrocarbons as the *Hevea* tree currently is in southeast Asia." More specifically, Adams and Seiler (1984) studied 48 accessions representing 39 taxa of sunflower (*Helianthus annuus* L., Asteraceae) to determine their potential for producing various compounds including NR. They concluded that "several sunflower species appear to offer an opportunity for the development of a whole-plant utilization crop based on multiple products such as hydrocarbons, natural rubber, specialty carbohydrates and livestock feed."

The sunflower genus *Helianthus* has 69 species and subspecies native to the U.S. Leaves of sunflower produce a small amount of NR, and, of the 53 species and subspecies of sunflower analyzed, 14 produced more than 0.93% NR (Stipanovic et al., 1982; Seiler et al., 1991). Those researchers postulated that there is a high genetic potential for increasing the NR content of cultivated sunflower. Twenty-eight taxa of sunflower were evaluated by Seiler et al. (1991), and of those, 13 species of wild sunflower contained NR and yielded at least 0.4% hydrocarbon. The molecular weight of the NR in sunflower was found to be low, but these researchers surmised that this low molecular weight NR might have potential use in some commercial applications. Numerous species of sunflower were evaluated in Texas for their NR content (Stipanovic et al., 1980). Two species of sunflower were found to contain 1.6% NR.

The ideal NR-producing crop would have several important characteristics (Pearson et al., 2007). Such a crop would be fast-growing with a high aerial biomass and partition a high percentage of its dry matter into the economic plant part that contains NR. Preferably, the crop would be an annual species that could be planted or rotated with other crops to meet changing market needs and conditions and the changing needs and conditions of specific farming operations. The ideal NR-producing crop must be suited for production using mechanized agriculture. The agronomy, such as planting, harvesting, soil fertility, pest control, and water requirements of the crop, needs to be well known to the farming community and farmers need to be acquainted with the production requirements of the crop. A NR-producing crop needs to be adapted to a wide range of environments, which will spread production risks related to the weather and other potential environmental problems. A NR-producing crop that is adapted to a wide range of environments would provide more farmers with an opportunity to grow the crop. Furthermore, a useful NR-producing crop for the U.S. would need to be grown on a relatively large number of acres to meet the United States NR needs. This would require the crop to be adapted to many of the northern climates (Buchanan et al., 1978).

Rather than relying on the one source of *Hevea* to meet the global demands for NR, multiple NR-producing crops would reduce risk and spread the production of NR across wider land areas. Growing more than one species of NR-producing crop would allow for greater flexibility to meet changing market conditions. Sunflower is a candidate as a source of NR because sunflower (1) already makes NR, which means it can compartmentalize this secondary product and the plant will likely do the same with larger endogenous amounts, (2) is adapted to agronomic production as an annual crop, (3) produces high biomass per hectare, (4) agronomy is well understood and would likely need only minor adjustment to be grown as a NR crop, (5) is adapted to mechanized agriculture, and (6) is a close relative of guayule (*Parthenium argentatum*) (Whitworth and Whitehead, 1991), a plant in which the biochemical regulation of NR yield and quality has been extensively studied. Both sunflower and guayule are in the tribe Heliantheae (this tribe is in the sub-family Asteroideae).

The objectives of this study were to: (1) evaluate commercial sunflower cultivars to determine biomass production and how they partition biomass into leaves, stems, and head, (2) determine how removing the head affects biomass partitioning, (3) determine latex concentration and yield in commercial sunflower cultivars and a diversity of genetic sunflower material, and (4) characterize several quality factors pertaining to the latex produced by these selected sunflower cultivars.

2. Materials and methods

2.1. Agronomic field experiments

Field performance tests were conducted at the Western Colorado Research Center at Fruita for three growing seasons to determine agronomic characteristics of commercially available sunflower cultivars for NR production. Seed of each of the sunflower cultivars was obtained from breeding/seed companies (Tables 1–3). The experiments were randomized complete blocks with four replications. Plot size was 0.4 m wide (4, 0.76 m beds) by 15.2 m long. The previous crops were alfalfa (*Medicago sativa* L.) in 2001 and corn (*Zea mays* L.) in 2002 and 2003. The soil type was a Youngston clay loam [fine-loamy, mixed (calcareous), and mesic Typic Torrifluvents]. Preplant fertilizer was applied each year at 23.7 kg N ha^{-1} and 112 kg P_2O_5 ha^{-1} using 11-52-0 and 45.5 kg N ha^{-1} of 32-0-0 was applied side-dress in 2001 and 32.3 kg N ha^{-1} of 32-0-0 was applied side-dress in 2002 and 2003 at approximately V8–V9 (Schneiter and Miller, 1981). Sunflower growth stages are designated as vegetative (V) and reproductive (R). The leaf number is determined by counting the number of true leaves starting at the base of the plant that are at least 4 cm in length and the numerical reproductive growth stages are noted when the sunflower plant reaches specified visible indicators of its ontogeny as documented by Schneiter and Miller (1981).

Pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzen amine] was applied each year preplant at 2.92 L ha^{-1} and incorporated in the upper 10 cm of the soil surface by two incorporation passes with shallow roller harrowing or with a spike-tooth harrow.

Planting occurred on 15 May 2001, 20 May 2002, and 22 May 2003. Plots were seeded each year using an air planter modified for planting small plots. Plant populations were determined at approximately V5–V7 (Schneiter and Miller, 1981) by counting plants along the entire length of the two center rows of each 4-row plot. An insecticide, cyhalothrin [[1α(S*),3α(Z)]-(±)-cyano-(3-phenoxyphenyl)methyl-3-(2-cholo-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropane-carboxylate], was applied according to the label for insect control in 2001. Sunflowers were furrow-irrigated with irrigation water from the Colorado River delivered through a canal system. Adequate irrigation water was available and was not a limiting factor for sunflower production in any year.

Harvest for aerial biomass occurred when plants reached R-8 (Schneiter and Miller, 1981). The two center rows of each 4-row plot were harvested with a commercial forage chopper. The forage mass was chopped and blown into a weigh truck that traveled alongside the forage chopper as plots were harvested. A subsample was obtained from each plot for moisture determination. Subsamples were weighed immediately after harvest and oven dried at 65 °C until constant weights were obtained. Aerial biomass was calculated on a dry matter basis.

Agronomic data were subjected to analysis of variance using Analytical Software Statistix 9 program (Analytical Software, 2008)

Table 1
Agronomic performance of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2001.

Cultivar	Brand/company	Biomass yield[a] (kg ha^{-1})	Plant water content at harvest (g kg^{-1})	Plant population (plants ha^{-1})
682	Triumph Seed	12,615	655	44,203
9501	Kaystar Seed	12,090	567	57,550
665	Triumph Seed	12,027	658	57,694
CL345	Croplan Genetics	11,264	604	61,568
573	Triumph Seed	11,072	671	57,407
4049	Interstate	10,581	661	59,415
567DW	Triumph Seed	10,576	594	54,106
RH 3733	Agway, Inc.	9978	611	54,823
Bronco	Seeds 2000	9538	600	62,573
766CRT	Triumph Seed	9425	589	50,661
652	Triumph Seed	8726	633	53,531
9404	Kaystar Seed	8437	582	55,253
CL385	Croplan Genetics	8218	569	52,096
8300	Kaystar Seed	8213	532	56,401
765C	Triumph Seed	7937	627	48,509
CL803	Croplan Genetics	6688	554	49,944
RH 3703	Agway, Inc.	6665	601	52,957
Avg.		9650	606	54,629
LSD (0.05)		2540	82	5907
CV (%)		18.5	9.6	7.6

[a] Calculated on a dry matter basis.

Table 2
Agronomic performance of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2002.

Cultivar	Brand/company	Biomass yield[a] (kg ha^{-1})	Plant water content at harvest (g kg^{-1})	Plant population (plants ha^{-1})
682	Triumph Seed	13,625	815	41,917
652	Triumph Seed	12,808	770	55,478
4049	Interstate	12,658	765	55,366
665	Triumph Seed	12,632	732	64,781
RH 3733	Agway, Inc.	12,417	741	49,762
573	Triumph Seed	11,860	737	55,142
765C	Triumph Seed	11,402	765	48,642
9501	Triumph Seed	11,354	772	48,418
766CRT	Triumph Seed	11,226	787	57,720
RH 3703	Agway, Inc.	11,197	754	48,417
Bronco	Seeds 2000	10,940	749	54,694
CL385	Croplan Genetics	10,823	749	53,909
9404	Kaystar Seed	9963	734	66,462
567DW	Triumph Seed	9734	744	66,126
8300	Kaystar Seed	9620	682	55,926
Avg.		11,484	753	54,851
LSD (0.05)		1802	53	12,780
CV (%)		11.0	49	16.3

[a] Calculated on a dry matter basis.

Table 3
Agronomic performance of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2003.

Cultivar	Brand/company	Biomass yield[a] (kg ha^{-1})	Plant water content at harvest (g kg^{-1})	Plant population (plants ha^{-1})
9501	Kaystar Seed	12,310	824	70,610
9404	Kaystar Seed	11,950	815	65,340
652	Triumph Seed	11,700	824	69,820
573	Triumph Seed	11,620	828	63,100
665	Triumph Seed	11,060	825	61,980
RH3733	Agway, Inc.	11,030	812	72,290
RH118	Kaystar Seed	10,900	819	65,450
682	Triumph Seed	10,640	835	57,160
765C	Triumph Seed	10,580	832	67,690
567DW	Triumph Seed	10,270	827	74,760
RH3703	Agway, Inc.	10,230	810	60,070
Bronco	Seeds 2000	10,170	833	61,870
766CRT	Triumph Seed	10,150	834	67,470
4049	Interstate	9902	834	64,000
CL345	Croplan Genetics	9755	837	75,200
CL385	Croplan Genetics	9595	830	63,880
CL803	Croplan Genetics	9523	809	60,410
8300	Kaystar Seed	8811	799	58,390
Avg.		10,565	824	65,527
LSD (0.05)		1517	19	8717
CV (%)		10.11	1.6	9.4

[a] Calculated on a dry matter basis.

to determine treatment (cultivar) effects. All statistical comparisons were conducted at the 5% level of probability using least significant difference method for mean separation.

Twenty sunflower lines representing a diversity of the genus *Helianthus* were obtained from Dr. Stephen Knapp at Oregon State University[5]. These diverse genetic materials were field planted in 2002 on the same day and other production practices were similar to the agronomic trial. These diverse genetic sunflower materials were used in latex quality analyses.

2.2. Biomass partitioning and manipulation

Biomass partitioning in sunflower was determined in 2002 and 2003 by sampling three plants at R-7 in each of the four replicates for each cultivar. Plants were weighed and separated into leaves, stems, and head and each of the three plant parts was oven dried at 65 °C, and weighed.

Biomass was manipulated by removing sunflower heads from ten consecutive plants of each cultivar in the four replicates at R-2. When plants reached R-8 the entire plant was hand-harvested at ground level and separated into leaves and stems. An additional ten consecutive plants with head intact were also harvested and separated into head, leaves, and stems.

2.3. Latex determination and quantification

2.3.1. Latex extraction and purification

Plants were hand-harvested and shipped overnight in coolers with ice packs (Koolit Gel Paks, catalog. no. 33500-585, vwr.com) from Fruita, CO to Albany, CA. Leaves were excised from the plants and ground in a one gallon Waring blender on low for 1 min in 0.2% NH_4OH, 0.1% Na_2SO_3, pH 10 (AAO) 1:1 (w:v). The homogenate was filtered through four layers of grade 60 cheesecloth. The filtrate was adjusted to pH 10 with NH_4OH and then to 200 mM EDTA. The homogenate was centrifuged for 25 min at 5850 × *g* in a bucket rotor. The floated latex layer was scooped off into 5 ml of AA0 and purified by repeated creaming in 0.1% ammonium alginate as described for *P. argentatum* latex (Cornish and Brichta, 2002). Creaming was repeated twice beyond when the subnatant became colorless. In some experiments, the plants were subdivided into leaves, stems, and flowers, and the flowers further subdivided into pappus and disc. Each plant part was homogenized and analyzed for latex content in a similar manner to the leaves.

2.3.2. Latex and rubber quantification

Thirty-five ml aliquots of homogenate were centrifuged for 25 min at 75,870 × *g* in a bucket rotor. Two ml of glacial acetic acid were pipetted onto the floated latex layer and the homogenate was recentrifuged. Coagulated latex was lifted off the homogenate onto tared weigh paper with a metal spatula, dried overnight at 37 °C, and weighed. Where the amount of latex was too small to be conveniently lifted from the tubes, the latex was collected, by vacuum-filtration, onto tared 0.25 μm cellulose acetate/cellulose nitrate filters, before drying and weighing.

Three leaves on the upper part of the plant were collected at R-8 in each plot of the 2002 sunflower agronomic field trial. Plants were frozen at −20 °C. Prior to analysis leaves were lyophilized (catalog. no. 7751000, Labconco Corporation, Kansas City, MO) and ground for approximately 90 s in a Wiley mill (Standard Model no. 3, Arthur H. Thomas Co., Philadelphia, PA) using a 2-mm mesh screen. Natural rubber and resin were extracted from plant tissue samples of four replicates using a Dionex (DIONEX Corporation, Sunnyvale, CA) Accelerated Solvent Extractor 200 (ASE) equipped with a Solvent Controller (DIONEX Corporation, Sunnyvale, CA). The ASE 200 was programmed and operated using a personal computer loaded with AutoASE 2.20 software (DIONEX Corporation, Sunnyvale, CA). All extractions with the ASE 200 were at 1500 psi. New Whatman 19.8 mm cellulose filters were inserted into 11-ml stainless steel extraction cells each time prior to loading samples into extraction cells.

Forty ml collection vials were used for collecting analyte. The amount of tissue sample for extractions ranged from 1.3 to 2.3 g. The extraction temperature for both acetone and hexane was 40 °C. Resin, i.e. lower molecular weight organic extract, was extracted in three, 25-min static cycles with acetone, and NR was extracted in two, 16-min static cycles with hexane. All acetone extractions were completed prior to extractions with hexane. Solvents containing analytes were evaporated from collection vials in a TurboVap LV evaporator (Zymark Corporation, Hopkinton, MA). Collection vials containing extracted analytes were oven dried at 56 °C and weighed to the nearest 1 mg. NMR (400 MHz) analysis of representative hexane fractions confirmed the composition of the analyte as *cis*-1,4-polyisoprene (Pearson et al., in press).

2.4. Rubber particle size analysis

The particle size distribution of purified, aqueous NR particle suspensions from aqueous extractions were determined using a LA900 laser light scattering particle size distribution analyzer (Horiba Instruments Inc., Irvine, CA) according to manufacture's instructions.

2.5. Latex rubber molecular weight characterization

Samples were prepared from creamed and coagulated sunflower latex. Coagulated latex (0.3–2.5 mg/ml) was weighed out into 8 ml glass vials with Teflon-lined caps. Tetrahydrofuran (THF) was added to the glass vials and used to dissolve the samples overnight. The dissolved samples were shaken and then 1 ml was filtered through 0.45 μm syringe filters composed of PTFE with GMF (Millipore 25 mm disposable GD/X filters) into autosampler vials with silicone/Teflon-lined septa. Each sample was run on an HP1100 series HPLC system connected to two downstream detectors using 100 μl per sample at a 1 ml/min flow rate for 17 min. The first detection system was a DAWN DSP Laser Photometer (Wyatt Technologies) containing 18 light scattering detectors and a 632.8 nm wavelength laser. An HP 1047 Refractive Index detector was connected to the DAWN via PEEK tubing with a delay volume of 0.167 ml. A PLgel 10 μm mixed-B size exclusion column (Hewlett Packard) and a phenogel 5 μm linear/mixed guard column (Phenomenex) were used with a column compartment temperature of 35 °C. The chromatograms were analyzed using Astra software (Wyatt Technologies). The resulting data from the chromatogram is used to determine number average molecular weight (Mn), weight average molecular weight (Mw), *z*-average molecular weight (Mz), and measure of polydispersity (Mw/Mn) (Flory, 1953).

3. Results

3.1. Agronomic field experiments

Because there was a significant year by sunflower cultivar interaction the agronomic data was analyzed separately by year. Biomass, averaged across all cultivars, was 9650, 11,484, and 10,565 kg ha^{-1} in 2001, 2002, and 2003, respectively (Tables 1–3). Biomass in 2001 ranged from a high of 12,615 kg ha^{-1} for cultivar 682 to a low of 6665 kg ha^{-1} for cultivar RH 3703. Biomass in 2002 ranged from a high of 13,625 kg ha^{-1} for cultivar 682 to a low

[5] Currently employed by Monsanto.

Table 4
Biomass partitioning of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2002.

Cultivar	Brand/company	Plant biomass[a] (g plant^{-1})	Leaf partitioning (%)	Stem partitioning (%)	Head partitioning (%)
682	Triumph Seed	314.6	22.6	37.3	40.2
9501	Kaystar Seed	305.5	19.5	36.8	43.7
766CRT	Triumph Seed	301.9	18.2	37.1	44.8
RH 3703	Agway, Inc.	252.8	15.9	35.8	48.4
4049	Interstate	250.7	18.8	40.1	41.1
765C	Triumph Seed	249.2	19.1	35.8	45.1
RH 3733	Agway, Inc.	243.1	15.9	36.2	48.0
665	Triumph Seed	243.0	19.9	36.9	43.3
573	Triumph Seed	225.6	19.5	38.2	42.3
652	Triumph Seed	223.8	19.5	36.2	44.3
Bronco	Seeds 2000	216.7	17.2	34.3	48.6
CL385	Croplan Genetics	199.9	18.2	34.3	47.5
8300	Kaystar Seed	185.3	15.7	30.7	53.7
9404	Kaystar Seed	171.4	16.1	40.0	44.0
567DW	Triumph Seed	168.7	24.1	31.9	44.0
Avg.		236.8	18.7	36.1	45.2
LSD (0.05)		92.4	3.5	4.6	5.6
CV (%)		27.3	13.0	8.9	8.7

[a] Calculated on a dry matter basis.

of 9620 kg ha^{-1} for cultivar 8300. Biomass in 2003 ranged from a high of 12,310 kg ha^{-1} for cultivar 9501 to a low of 8811 kg ha^{-1} for cultivar 8300. There were significant differences among sunflower cultivars for biomass in each of the 3 years. Seven cultivars produced significantly more biomass than other sunflower cultivars in 2001 and 2003. Six cultivars in 2002 produced more biomass than other cultivars in 2002.

Plant water content at harvest, averaged across all cultivars, was 606, 753, and 824 g kg^{-1} in 2001, 2002, and 2003, respectively (Tables 1–3). Plant water content in 2001 ranged from a high of 671 g kg^{-1} for cultivar 573 to a low of 532 g kg^{-1} for cultivar 8300. Plant water content in 2002 ranged from a high of 815 g kg^{-1} for cultivar 682 to a low of 682 g kg^{-1} for cultivar 8300. In 2003, plant water content ranged from a high of 837 g kg^{-1} for CL345 to a low of 799 g kg^{-1} for 8300. There were significant differences among sunflower cultivars for plant moisture in each of the 3 years. Differences among sunflower cultivars for water content may be due, in large part, to maturity differences.

Plant population, averaged across all cultivars, was 54,629, 54,851, and 65,527 plants ha^{-1} in 2001, 2002, and 2003, respectively (Tables 1–3). Plant populations in 2001 ranged from a high of 62,573 plants ha^{-1} for Bronco to a low of 44,203 plants ha^{-1} for cultivar 682. Plant populations in 2002 ranged from a high of 64,781 plants ha^{-1} for 665 to a low of 41,917 plants ha^{-1} for 682. Plant populations in 2003 ranged from a high of 75,200 plants ha^{-1} for CL345 to a low of 57,160 plants ha^{-1} for cultivar 682. There were significant differences among sunflower cultivars for plant population in each of the 3 years. Differences in plant populations may be due in part to the quality of the seed at the time of planting. Additionally, plant populations may also be affected by how well the plot planter can operate when planting seed of sunflower cultivars that have different seed sizes.

3.2. Biomass partitioning and manipulation

Sunflower plant biomass was determined on a per plant basis. The plant biomass data in Tables 4 and 5 are presented on a mass basis while the data for partitioning of leaf, stem, and head are presented as a percentage of the total plant biomass. There were significant differences among sunflower cultivars in each of the 2 years for plant biomass, and for leaf, stem, and head partitioning.

Table 5
Biomass partitioning of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2003.

Cultivar	Brand/company	Plant biomass[a] (g plant^{-1})	Leaf partitioning (%)	Stem partitioning (%)	Head partitioning (%)
765C	Triumph Seed	342.0	16.4	38.7	45.0
665	Triumph Seed	260.3	17.4	41.1	41.6
RH3703	Agway, Inc.	259.5	15.6	38.0	46.3
RH118	Agway, Inc.	257.3	14.5	40.5	45.1
652	Triumph Seed	240.0	15.6	39.5	44.9
9404	Kaystar Seed	239.7	15.8	39.7	44.5
766CRT	Triumph Seed	229.1	19.2	41.6	39.1
CL385	Croplan Genetics	225.6	19.2	38.5	42.3
682	Triumph Seed	221.3	18.4	47.6	34.0
9501	Kaystar Seed	218.3	15.8	42.5	41.6
573	Triumph Seed	215.2	16.0	40.9	43.1
4049	Interstate	214.2	15.2	42.9	42.0
RH3733	Agway, Inc.	213.0	14.5	41.0	44.5
567DW	Triumph Seed	187.4	23.8	33.4	42.8
Bronco	Seeds 2000	171.4	18.8	40.1	41.1
CL803	Croplan Genetics	171.1	14.9	34.3	50.8
CL345	Croplan Genetics	167.6	16.0	39.0	44.9
8300	Kaystar Seed	139.9	14.3	34.7	51.0
Avg.		220.7	16.7	39.7	43.6
LSD (0.05)		101.8	3.8	4.0	5.7
CV (%)		32.5	16.2	7.1	9.2

[a] Calculated on a dry matter basis.

Plant biomass, averaged across all cultivars, was 236.8 g plant^{-1} in 2002 and 220.7 g plant^{-1} in 2003 (Tables 4 and 5). Plant biomass in 2002 ranged from a high of 314.6 g plant^{-1} for 682 to a low of 168.7 g plant^{-1} for 567DW. Plant biomass in 2003 ranged from a high of 342.0 g plant^{-1} for cultivar 765C to a low of 139.9 g plant^{-1} for cultivar 8300. Ten of the fifteen sunflower cultivars evaluated produced more plant biomass than the other five cultivars.

Leaf partitioning, averaged across all cultivars, was 18.7% in 2002 and 16.7% in 2003 (Tables 4 and 5). Leaf partitioning in 2002 ranged from a high of 24.1% for cultivar 567DW to a low of 15.7% for cultivar 8300. Leaf partitioning in 2003 ranged from a high of 23.8% for cultivar 567DW to a low of 14.3% for cultivar 8300.

Stem partitioning, averaged across all cultivars, was 36.1% in 2002 and 39.7% in 2003 (Tables 4 and 5). Stem partitioning in 2002 ranged from a high of 40.1% for cultivar 4049 to a low of 30.7% for cultivar 8300. Stem partitioning in 2003 ranged from a high of 47.6% for cultivar 682 to a low of 33.4% for 567DW.

Head partitioning, averaged across all cultivars, was 45.2% in 2002 and 43.6% in 2003 (Tables 4 and 5). Head partitioning in 2002 ranged from a high of 53.7% for cultivar 8300 to a low of 40.2% for cultivar 682. Head partitioning in 2003 ranged from a high of 51.0% for cultivar 8300 to a low of 34.0% for cultivar 682. Differences among sunflower cultivars for biomass partitioning may be due, in part, to maturity differences.

Biomass manipulation was accomplished in 2001 by removing the head from sunflower at R-2 to determine how the sunflower plant would partition biomass with the head removed. The total biomass of many sunflower cultivars was increased when the head was removed (Table 6). As would be expected, removing the head increased biomass partitioning to leaves and stems. A greater increase of partitioning occurred in stems than in leaves. On a per plant basis, total biomass of sunflower plants with heads intact was highest for 682 and least for 8300 (Table 6). Averaged across all cultivars, total biomass was 547 g plant^{-1} (leaves had 84 and stems had 213 g plant^{-1}).

Total biomass of sunflower plants with heads removed was also highest for 682 and least for 8300 (Table 6). Averaged across all cultivars, total biomass was 632 g plant^{-1} (leaves had 208 and stems had 423 g plant^{-1}). Thus, Triumph 682 produced the most biomass with and without heads and Kaystar 8300 produced the least amount of biomass with and without heads.

Removing the head did not affect sunflower cultivars similarly. Some sunflower cultivars were triggered to produce substantially more secondary reproductive structures than others (Table 6). Some sunflower cultivars produced only a few secondary reproductive structures. Cultivar 682 only produced two secondary reproductive structures and 567DW did not produce any secondary reproductive structures.

3.3. Latex determination and quantification

We attempted to extract and quantify latex in leaves, stems, flowers, pappus, and disc of sunflower. No latex was found in mature stems or in the pappus of the flowers. The latex was found almost entirely in the leaves of young and mature sunflowers. Observations suggested that a small amount of latex might be present in the flower discs but the presence of a large number of oil bodies made it impossible to accurately or reproducibly assess latex content in this tissue.

Considerable variation was apparent in the amount of latex produced by leaves of different sunflower cultivars, in the agronomic field trial, ranging from only 0.97 mg g^{-1} in 652 to 6.07 mg g^{-1} in 9404 (Table 7). When ranked in order of latex yield three groupings were apparent. Nine cultivars contained a similar amount of latex in the middle grouping. Three cultivars comprised the lower grouping and five cultivars had higher latex yields than the other two groupings (Fig. 1). The diverse genetic sunflower material showed a very similar range of leaf latex content to the cultivars in the agronomic trial (Fig. 2), ranging from 1.40 mg g^{-1} HA370 to 6.32 mg g^{-1} in PHD. Stipanovic et al. (1980, 1982) found the NR content of wild strains of *Helianthus* to range from only trace amounts to as high as 16 mg g^{-1}.

Deheading plants of eight cultivars in the agronomic trial, spanning the full range of latex contents, only markedly increased leaf latex content in the lowest yielding cultivar, 652, had little effect in most cultivars, and greatly reduced latex content in the two highest NR yielding cultivars, 4049 and 9404 (Fig. 3). Latex concentration of sunflower plants with heads removed was highest for 8300 at 4.00 mg g^{-1} of leaf and least for 765C at 1.31 mg g^{-1} of leaf material for those cultivars in which latex concentration was determined.

Given the latex concentration in the leaf and dry matter production per hectare, latex yield per hectare of sunflower plants with heads intact was highest for cultivar 665 at 30.86 kg ha^{-1} and least

Table 6
Biomass[a] manipulation of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2001.

Cultivar	Brand/company	Plants w/heads Total biomass (g plant^{-1})	Plants w/heads Leaves (g plant^{-1})	Plants w/heads Stems (g plant^{-1})	Plants w/o head Total biomass (g plant^{-1})	Plants w/o head Leaves (g plant^{-1})	Plants w/o head Stems (g plant^{-1})	Secondary reproductive structures (g plant^{-1})
682	Triumph Seed	923	164	345	1098	392	706	2
665	Triumph Seed	654	157	212	882	316	566	14
RH 3703	Agway, Inc.	651	52	191	493	134	359	14
765C	Triumph Seed	638	66	251	582	161	421	9
Bronco	Seeds 2000	576	91	246	681	235	446	133
RH 3733	Agway, Inc.	572	53	194	449	129	320	17
573	Triumph Seed	559	85	247	622	210	412	6
766CRT	Triumph Seed	553	90	203	664	220	445	12
4049	Interstate	546	99	257	665	207	458	127
CL385	Croplan Genetics	542	88	225	599	215	384	123
567DW	Triumph Seed	513	103	167	663	262	401	0
9501	Kaystar Seed	507	76	253	715	235	479	32
652	Triumph Seed	491	54	185	497	139	359	9
CL345	Croplan Genetics	488	72	206	522	142	381	14
9404	Kaystar Seed	461	78	178	764	273	491	7
CL803	Croplan Genetics	317	63	141	490	166	325	19
8300	Kaystar Seed	301	46	114	357	110	246	34
Avg.		547	84	213	632	208	423	34
LSD (0.05)		201	43	83	228	87	149	35
CV (%)		25.8	35.8	27.3	25.4	29.3	24.7	72.4

[a] Calculated on a dry matter basis.

Table 7
Latex production of sunflower cultivars grown at the Western Colorado Research Center at Fruita during 2001.

Cultivar	Brand/company	Latex concentration			
		w/head (mg g^{-1} dw leaf)	w/o head (mg g^{-1} dw leaf)	w/head (kg ha^{-1})	w/o head (kg ha^{-1})
682	Triumph Seed	1.37	1.55	9.96	26.88
9501	Kaystar Seed	2.11	–	9.22	–
665	Triumph Seed	3.41	3.13	30.86	64.47
CL345	Croplan Genetics	1.71	–	7.56	–
573	Triumph Seed	2.98	–	14.53	–
4049	Interstate	4.92	3.61	28.94	44.36
567DW	Triumph Seed	1.75	2.14	9.74	30.34
RH 3733	Agway, Inc.	1.81	–	5.26	–
Bronco	Seeds 2000	2.10	–	8.87	–
766CRT	Triumph Seed	1.94	–	8.85	–
652	Triumph Seed	0.97	2.87	2.79	21.35
9404	Kaystar Seed	6.07	1.19	26.14	17.96
CL385	Croplan Genetics	2.32	–	10.63	–
8300	Kaystar Seed	3.90	4.00	10.11	24.81
765C	Triumph Seed	2.30	1.31	7.36	10.23
CL803	Croplan Genetics	1.70	–	5.34	–
RH 3703	Agway, Inc.	1.13	–	3.11	–

for cultivar 652 at 2.79 kg ha^{-1} (Table 7). Stipanovic et al. (1980) estimated NR yields for sunflower at approximately 22 kg ha^{-1}. Latex yield per hectare of sunflower plants with heads removed was highest for 665 at 64.47 kg ha^{-1} and least for 765C at 10.23 kg ha^{-1}.

Average NR determination of the 15 cultivars was 12.64 mg g^{-1} (Fig. 4). The amount of rubber extracted by solvent with the ASE at sunflower growth stage R9 was higher (2–10-fold) than the amount of latex obtained from the same cultivars aqueous extraction, by aqueous extraction, suggesting that latex extraction was relatively inefficient. Post-harvest rubber coagulation could render some fraction of the rubber inaccessible to water extraction, as for example in guayule (Coffelt et al., 2009). Also, some rubber particles may have been too heavy to recover by the latex extraction method used, but would be accessible to the solvent extraction ASE procedure. Natural rubber of the 15 cultivars ranged from a high of 16.30 mg g^{-1} for CL385 to a low of 9.03 mg g^{-1} for 682 (Fig. 4). Six sunflower cultivars had higher NR contents than those of other cultivars, namely CL385, 567DW, Bronco, 4049, 665, and 9404. Both 4049 and 9404 were found to have both higher NR and latex yields than other cultivars.

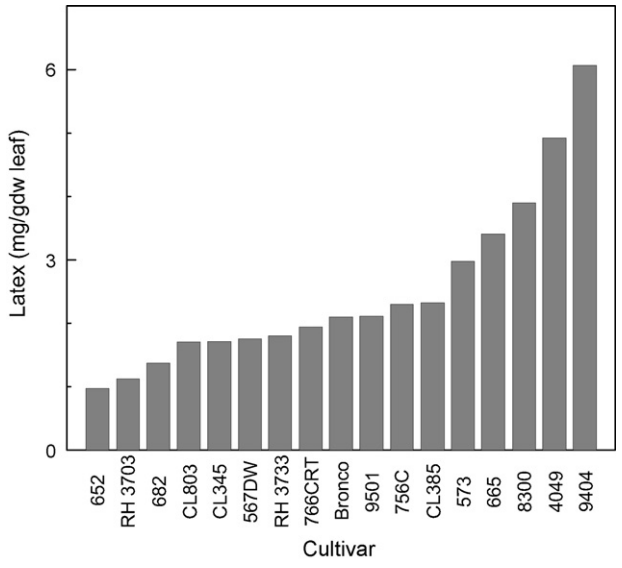

Fig. 1. Latex yield from leaves of sunflowers grown in Colorado. Based on the capacity of the procedure and the size of the tissue sample that could be processed and the low concentration of latex in sunflower, only one replicate was possible, and thus, no errors bars are presented.

3.4. Rubber particle size analysis

The light and heavy particles from two lines were compared but little difference was found between them (Fig. 5). The range in particle size of the sunflower lines grown in the agronomic trial and in the diversity trial was similar (Fig. 6) but a substantial variation in particle size distribution was seen among lines, with mean particle diameters from 1.29 to 2.26 μm.

3.5. Latex rubber molecular weight characterization

Sufficient latex was purified from cultivars 573 and 4049 for molecular analysis. It was immediately apparent that the molecular weight of the NR produced was bimodal (Fig. 7) although the relative abundance of the two peaks differed in the two cultivars, there being more of the first (higher molecular weight) peak in 573 than in 4049. When the differential molar mass was plotted and

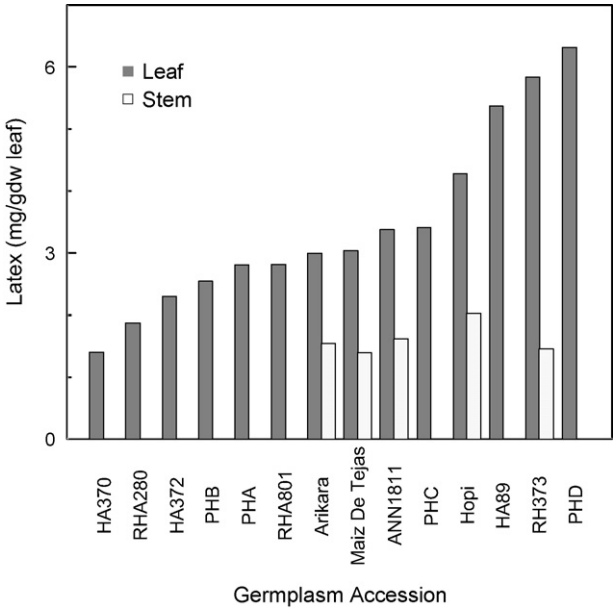

Fig. 2. Latex yield from leaves and stems of sunflowers grown in the diversity trial. Stem values were only determined for a subset of cultivars as shown. Based on the capacity of the procedure and the size of the tissue sample that could be processed and the low concentration of latex in sunflower, only one replicate was possible, and thus, no errors bars are presented.

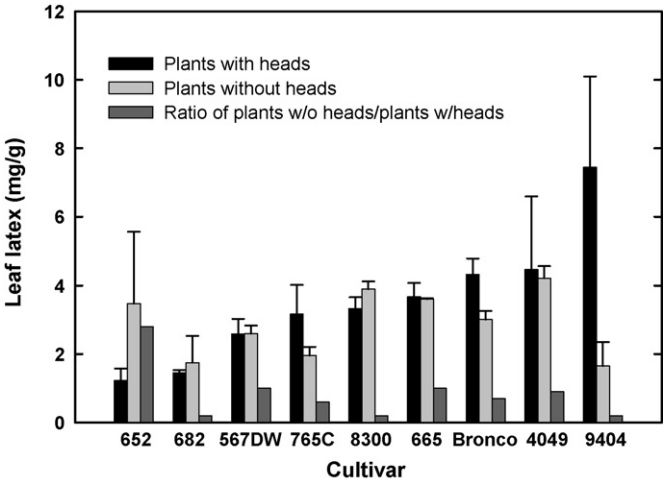

Fig. 3. Effect of deheading on latex yield of sunflowers grown at Fruita, Colorado. Standard errors of two replicates are shown for each sunflower cultivar.

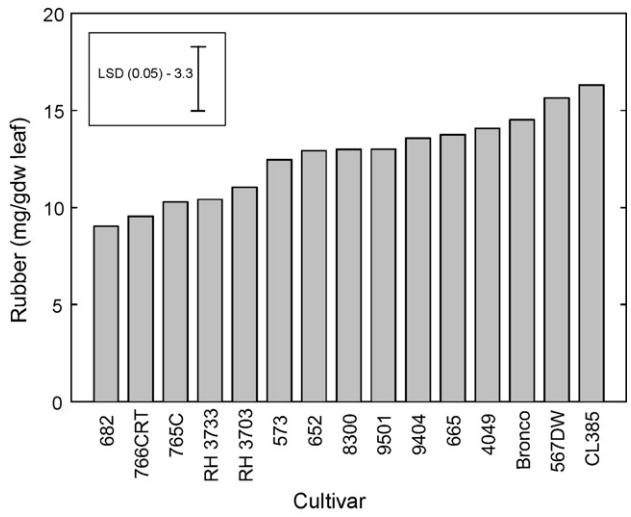

Fig. 4. Natural rubber that was solvent extracted from leaves of sunflower cultivars grown at Fruita, Colorado during 2002.

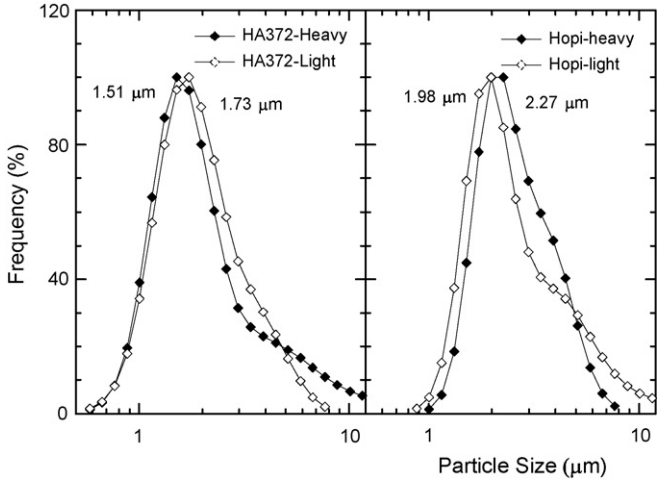

Fig. 5. A comparison of the particle size distribution of two subfractions of sunflower rubber particles differing in specific gravity.

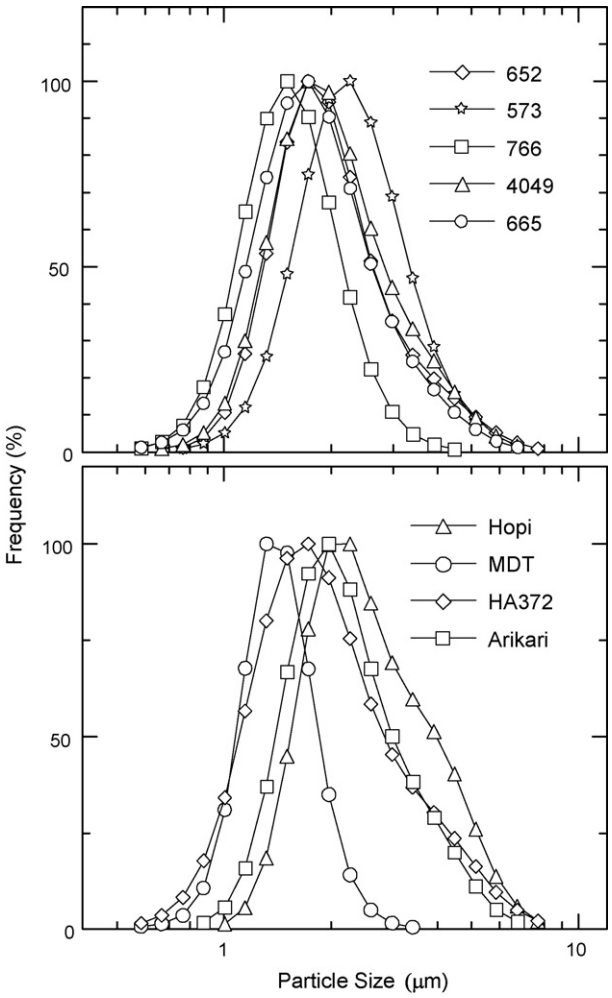

Fig. 6. Rubber particle size distribution in latex purified from sunflower cultivars grown in the agronomic trial (upper panel) and sunflower diversity trial (lower panel). MDT = Maiz de Tejas.

compared with latex NR from *H. brasiliensis* and *P. argentatum* it was apparent that the sunflower NR samples had very little high molecular weight material (Fig. 8). Also, a very similar chromatograph was observed for all 18 light scattering angles (Fig. 9) for the low molecular weight peak indicating a lack of branching and/or crosslinking. In *H. brasiliensis*, which makes branched NR polymers, the comparable plot shows an 80% diminution in peak height from angles 1 to 18 (data not shown).

Molecular characterization parameters (Table 8) showed that the NR was consistent among replicates from each sunflower cultivar and that the larger molecular peak clearly seen in the chromatogram (Fig. 7) accounted for only 3 or 5% of the total amount of NR in 4049 and 573, respectively (see also Fig. 8).

4. Discussion

4.1. Agronomic field experiments

The success of NR production, as with other crop production enterprises, is highly dependent on yield per area and cultivars selected for planting can have a significant impact on yield. Some sunflower cultivars produced significantly more biomass yield than others. Sunflower cultivars 665 and 573 were high yielding in all 3 years while 9501, 652, RH3733, 4049 were high yielding in two of the 3 years. Plant water content at harvest varied considerably

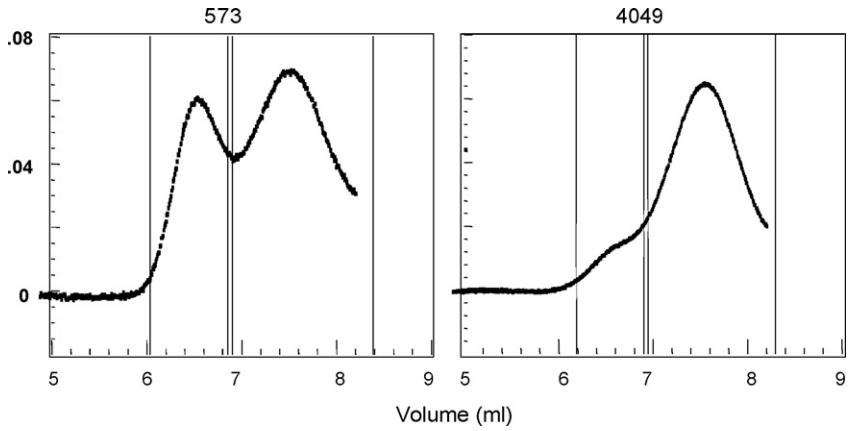

Fig. 7. Size exclusion chromatogram of latex rubber purified from two sunflower cultivars grown at Fruita, Colorado.

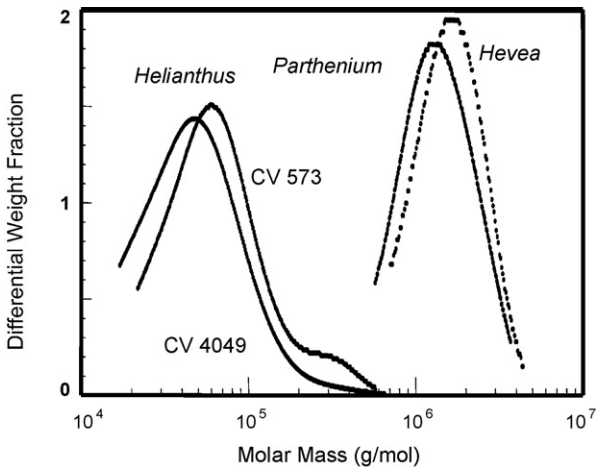

Fig. 8. Molecular weight profile of latex rubber purified from two sunflower cultivars grown in Colorado compared with latex rubber purified from *Hevea brasiliensis* and *Parthenium argentatum*.

with sunflower cultivar and with year. The preferred plant moisture content at harvest for sunflowers grown for NR production is not known and would have to be determined through additional study. However, sunflowers grown for latex production, in contrast to solid rubber, would need to be sufficiently hydrated at harvest to maintain the latex particles in aqueous suspension during processing. Plant populations for the sunflower cultivars evaluated in this trial varied by cultivar and year. There did not appear to be a correlation between plant population and biomass yield of sunflower.

4.2. Biomass partitioning and manipulation

High biomass yields would be important for NR production particularly if the sunflower plant partitioned more of its biomass into leaves rather than other plant parts. Not surprisingly, given the years of plant development for seed production, most of the sunflower biomass is partitioned into head and stem. A large head is needed for production of a large quantity of seed and a substantial stem is needed to support a large head. Sufficient leaf material is only needed to photosynthesize biomass to meet the needs of the plant, given how the sunflower plant currently allocates resources.

Sunflower leaves are of particular interest because they contain higher NR concentrations than other plant parts. In both years evaluated, 567DW, a dwarf sunflower type, partitioned more of its dry matter into leaves than any other cultivar; however, it was not among the cultivars that produced a large amount of biomass. In fact, in 2002, 567DW produced the least amount of biomass of all the cultivars. This indicates that with plant breeding/genetic manipulation it may be possible to develop sunflower cultivars that

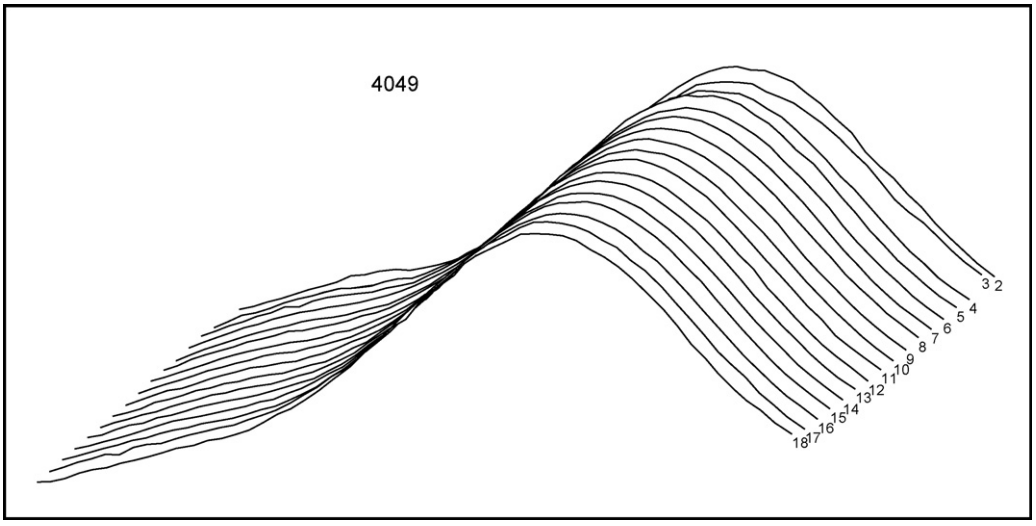

Fig. 9. Size exclusion data across 18 light scattering angles for latex rubber purified from cultivar 4049, grown at Fruita, Colorado.

Table 8
Molecular characterization of latex rubber purified from two *Helianthus annuus* cultivars compared with latex rubber from *Parthenium argentatum* and *Hevea brasiliensis*. Each *H. annuus* value is the mean of 4 ± S.E. Mn = number average molar mass, Mw = weight average molar mass, Mz = z-average molar mass, Mw/Mn = polydispersity (1 = monodispersed).

Sample	Peak # and S.E.	Mass in peak (%)	Mn	Mw	Mz	Mw/Mn
H. annuus						
cv 573	1	95	64,400	73,800	84,500	1.146
	S.E.		2930	2720	2420	0.013
	2	5	518,700	568,600	652,200	1.115
	S.E.		35,300	39,200	45,200	0.007
cv 4049	1	97	54,500	66,600	83,500	1.228
	S.E.		2720	2050	3450	0.039
	2	3	448,100	492,200	551,400	1.094
	S.E.		46,500	60,100	81,900	0.020
P. argentatum			1,074,950	1,333,000	1,644,000	1.240
H. brasiliensis			1,348,000	1,686,000	2,053,000	1.251

partition more of their biomass into leaves rather than other plant parts while still maintaining a plant that is suitable for agriculture.

Sunflower cultivars with the highest and lowest biomass production maintained the same ranking among the cultivars regardless if the head was intact or removed. With many sunflower cultivars, removing the head increased plant weight and stem weight. As would be expected with the head removed, energy produced that would normally go to the head was partitioned into larger leaves and stems. This again indicates that plant breeding for increased sunflower latex production should seek to select for smaller heads and increased biomass production of leaves. Given the cultivars included in our study, it appears that some cultivars would be more responsive to breeding for increased leaf mass than other cultivars.

4.3. Latex determination and quantification

The quantity of NR latex produced by sunflower in our studies was similar to those previously reported in the literature (Bowers, 1990). The potential for increasing latex production in sunflower appears possible, given current levels are low and reasonable advances in latex production in sunflower plants through plant breeding and genetic engineering could be achieved. However, it also is clear that although head removal generally increased the amount of NR-containing leaf, the concentration of NR in the leaves was severely reduced by deheading in cultivars 9404 and 765C; 9404 was transformed by deheading from having the highest leaf NR content to the lowest of all the cultivars. Remarkably, a single cultivar, 652, responded to deheading by increasing the concentration of NR in its leaves 3-fold, again highlighting considerable genetic variation in NR yield.

4.4. Rubber particle size analysis

Heavy and light NR particles, as found in sunflower (Fig. 5), also are found in *Ficus elastica* (Cornish and Siler, 1996), although even these "light" particles have a specific gravity of >1. There was no size difference apparent between the two types in *F. elastica* as is the case for sunflower. However, enzyme activity was confined to the light *F. elastica* particles. Heavy particles are not apparent in *P. argentatum* or *H. brasiliensis*, although it is possible that density subsets exist, but that both have specific gravities of below 1 and so would not be detected using our aqueous methods. The molecular weight of NR in *F. elastica* was the same in heavy and light particles. Searching for commonalities, *F. elastica* and *H. annuus* (HA372 and Hopi), both have particles larger than *H. brasiliensis* and *P. argentatum*, and both make significant amounts of low molecular weight NR. It is not clear how these differences may relate to the density differences.

4.5. Latex rubber molecular weight characterization

The bimodal molecular weight of the NR produced by sunflowers indicates that sunflower is capable of biosynthesizing quite high molecular weight NR. In previous research conducted by Seiler et al. (1991), the molecular weights of several taxa of *Helianthus* ranged from 29.8×10^3 to 73.3×10^3. Swanson et al. (1979) reported that the molecular weight of NR in *H. hirsutus* was 2.79×10^5. Our results revealed that sunflower is capable of synthesizing small amounts of higher molecular weight NR of approximately 600,000 g/mol.

Cultivar 573 produced a considerably higher molecular weight NR than 4049, and even its lower molecular weight NR was significantly higher than the low molecular weight NR in 4049. It does not seem likely that sunflower possesses two NR polymerizing enzymes each making a different polymer length. However, *in vitro* experiments with purified enzymatically active NR particles has made it clear that polymer molecular weight is readily altered by the relative concentration of the initiating (allylic pyrophosphate, APP) and polymerizing (isopentenyl pyrophosphate, IPP) substrates for NR biosynthesis, and high molecular weight NR is made only when there is an excess of IPP and when the APP pool is limiting (Cornish et al., 2000). These substrates also are key building blocks for the isoprenoid pathway, which is essential in plant growth and development. The concentration of magnesium ions, an essential cofactor for many isoprenoid enzymes, also directly impacts NR biosynthetic rate and molecular weight (da Costa et al., 2004). The availability of these substrates and cofactor in the leaf for NR biosynthesis will undoubtedly change during leaf development.

Sunflower cultivars exhibited considerable genetic variation for biomass partitioning and NR yield and quality. Focused plant breeding and genetic manipulation will be required to develop novel sunflower cultivars suitable for commercial production of NR.

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