Seed Yield, Oil, and Fatty Acids of Cuphea in the Northwestern Corn Belt

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ABSTRACT

Cuphea is a potential new crop for temperate regions. It produces and stores in its seeds medium chain length fatty acids, which currently are derived commercially from seeds of tropical palms. The growth and yield potential of 'PSR23' (*Cuphea viscosissima* Jacq. × *C. lanceolata* W.T. Aiton) cuphea was known for west central Minnesota but not elsewhere. To better understand the range of latitudes in which PSR23 is adapted, planting date experiments were established at seven research farms along a transect from southwestern Iowa to northwestern Minnesota (41–49° N latitude) in 2002 and 2003. Seed yields, seed oil contents, and fatty acid profiles were determined. In the absence of drought, cuphea grew well vegetatively at most sites, but seed yields tended to be higher in Minnesota than in Iowa. Irrigation did not enhance seed yields greatly in Iowa. Low yields due to delayed planting (mid May to mid June) were apparent only when water was limited. Oil content of seeds ranged from 28 to 33% and may have been associated inversely with air temperatures during seed-fill. The principal fatty acid was capric acid, which ranged from 67 to 73% of total oil and was always highest in the colder, northern-most sites. PSR23 appears to have better potential as an industrial oilseed crop at higher than lower latitudes because of enhanced yields and capric acid levels.

COCONUT and oil palm (*Cocos nucifera* L. and *Elaeis guineensis* Jacq.) currently are the sole sources of the world's supply of plant-derived medium chain length fatty acids (MCFA), such as capric, lauric, and myristic acids. These fatty acids are used in detergents, lubricants, cosmetics, and confectionary products. Temperate sources of MCFA would be advantageous to both industry and agriculture based at higher latitudes. *Cuphea* (henceforth, "cuphea"), which is a genus of some 260 species, is a potential source of such fatty acids in temperate growing regions (Graham and Knapp, 1989; Hirsinger, 1985; Knapp, 1993).

Although some species of cuphea are used in ornamental horticulture, and several species have been examined for agronomic potential (Hirsinger, 1985), the interspecific hybrid known as PSR23 (Knapp and Crane, 2000a) currently is the source of most agronomic studies with this taxon. PSR23 is a cross between the North American native, summer annuals, *Cuphea viscosissima* Jacq. and *C. lanceolata* W.T. Aiton. Selection following hybridization resulted in plants whose seed dormancy, seed retention, and self-fertility characteristics were agronomically superior to those of the parental species. Nevertheless, PSR23 still can be considered only partially domesticated. Remaining obstacles include an indeterminate growth habit, continued seed shattering, and only partial self-fertility.

PSR23 cuphea grows well in west-central Minnesota (45°35′ N latitude), with yields capable of exceeding 1000 kg ha^{-1}, when it is planted in early to mid May (Gesch et al., 2002b), at densities of about 1 to 2 million plants ha^{-1} (Gesch et al., 2003), harvested in late September (Gesch et al., 2005), and provided with sufficient rainfall or irrigation to maintain relatively high plant available water throughout the growing season (Sharratt and Gesch 2004). Little is known about seed yield potential of PSR23 grown elsewhere. The only other published report for seed yield of PSR23 is from its developers (Knapp and Crane, 2000a), who listed a seed yield of 795 kg ha^{-1} for an irrigated site at Corvallis, OR (44°34′ N). Seed yields of PSR23 cuphea grown near Peoria, IL (40°45′ N), have not been as high as those from Minnesota (personal observations).

The earlier 'IH50' hybrid of *C. viscosissima* × *lanceolata* was tested by Roath (1998) at Ames, Iowa (42°00′ N). Seed yields ranged widely, from 220 kg ha^{-1} in 1993 to 750 kg ha^{-1} in 1994. For comparison, adjacent plots of *C. lanceolata* 'LN86' produced from 200 to 660 kg ha^{-1} of seed. Other experiments with *C. lanceolata* LN86 (Roath, 1998) and 'LN43' (Knapp and Crane, 2000a; Webb and Knapp, 1991) indicated that seed yield potential was low compared with that for PSR23, as was that for *C. viscosissima* 'VL90' (Knapp and Crane, 2000a).

Hirsinger (1985) performed agronomic trials at Davis, CA (38°35′ N) and Corvallis, OR, on several cuphea species, including *C. lanceolata* and *C. viscosissima*, but reported seed yields only in terms of grams per plant. He extrapolated these data to suggest that maximum unit-area seed yield in his experiments was 900 kg ha^{-1} (Hirsinger, 1985, p. 80), but he did not indicate the species, site, and growing conditions associated with this suggestion. No other published unit-area seed yields for PSR23 cuphea, or its parents, are known by our team.

Seeds of PSR23 contained about 30% oil when plants were grown in the Willamette Valley of Oregon (Knapp and Crane, 2000a), a location whose average daily air temperature during September (T_{Sep}), the presumed time of seed-fill, is 16.4°C. In contrast, PSR23 seeds typically contained ≤28% oil when grown in west central Minnesota (Gesch et al., 2002b, 2003), where T_{Sep} is 15.0°C. When PSR23 was allowed to mature fully (late September harvest), seeds contained >32% oil in a year when T_{Sep} was high (17°C), but <30% oil when T_{Sep} was low (15°C) (Gesch et al., 2005). This information suggests

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Abbreviations: FAME, fatty acid methyl ester; GDD, growing degree days; GS, gas chromatography; MCFA, medium chain length fatty acid; PSR23, partial seed retention line #23; T_{Sep}, average daily September air temperature.

that oil synthesis or storage in PSR23 cuphea may increase with temperature.

Seed oil levels of other oilseed species are correlated with temperature during the period of seed-fill, but the nature of the reported relationships can be positive or negative (e.g., Fieldsend and Morison, 2000; Green, 1986; Kane et al., 1997; Thomas et al., 2003; Yaniv et al., 1989). A logical interpretation of these fragmented data is that a quadratic-type response best describes the effect of temperature during seed-fill on seed oil percentage, e.g., the temperature maximum in soybean [*Glycine max* (L.) Merr.] is about 28°C, below or beyond which oil levels decrease (Piper and Boote, 1999), whereas that for sunflower (*Helianthus annuus* L.) is about 21°C (Canvin, 1965). Differences in seed oil content can be appreciable from plants grown in differing environments. For instance, the same six soybean lines averaged 27% (±8%) more oil in seeds from plants grown in Mississippi (33°25′ N latitude), where seed-fill temperature was 26.9°C, than those from Indiana (40°25′ N latitude), with a seed-fill temperature of 21.4°C (Cherry et al., 1985).

Although the small differences in seed oil content caused by environment are seemingly trivial, they can have considerable impacts on industrial efficiency for oil extraction. Thus, understanding effects of climate and its manageable correlates, plating date and location, on seed oil production is important for a potential new crop such as PSR23 cuphea.

The predominant fatty acid of both *C. viscosissima* and *C. lanceolata* is capric acid, which constitutes about 76 and 83%, respectively, of total oil (Graham and Knapp, 1989) for wild-type plants. Capric acid also is the major fatty acid in PSR23, averaging about 70% (Isbell and Behle, 2003), but variability surrounding this average is unknown. Concentrations of some fatty acids vary according to air temperature during seed-fill. For instance, oleic acid often increases with increasing temperature, whereas linoleic and/or linolenic decrease (Kane et al., 1997; Sarmiento et al., 1998; Thomas et al., 2003; Wilcox and Cavins, 1992; Yaniv et al., 1989). Thus, because plating date can influence the timing of seed-fill and, therefore, temperatures during seed-fill, it represents a potential management tool to manipulate fatty acids profiles. Similarly, selection of growing regions with differing climates during seed-fill also embodies a choice, albeit on a larger scale, for managing fatty acid levels in oilseeds.

Our objectives were to examine the adaptation of PSR23 cuphea in terms of seed yield, seed oil content, and MCFA profile along a latitudinal transect of research farms from southwestern Iowa to northwestern Minnesota. Because length of growing season and air temperatures during seed maturation are intimately associated with latitude, planting date also was studied at each experimental site as a potential means of manipulating growing season and the seed-fill climate.

MATERIALS AND METHODS

Experiments were conducted at seven research farms, all of which were situated along a 1000 km transect at about 96° W longitude (Table 1). Each farm was associated either with Iowa State University, the University of Minnesota, or USDA-ARS. These farms were the Armstrong Research and Demonstration Farm near Lewis, IA; the Western Research and Demonstration Farm near Castana, IA; the Northwest Research and Demonstration Farm near Calumet, IA; the Southwest Research and Outreach Center near Lamberton, MN; the Swan Lake Research Farm near Morris, MN; the Northwest Research and Outreach Center near Crookston, MN; and the Magnusson Research Farm near Roseau, MN. Soils of the seven experimental sites, in the same order as above, were: Marshall silty clay (Typic Hapludoll, fine-silty, mixed, superactive, mesic), Manona silty clay loam (Typic Hapludoll, fine-silty, mixed, superactive, mesic), Sac silty clay (Oxyaquic Hapludoll, fine-silty, mixed, superactive, mesic), Normania clay loam (Aquic Hapludoll, fine-loamy, mixed, superactive, mesic), Barnes loam (Calcic Hapludoll, fine-loamy, mixed, superactive, frigid), Wheatville loam (Aeric Calciaquoll, coarse silty over clayey, mixed smectitic, superactive, frigid), and Percy sandy clay loam (Typic Calciqauoll, fine-loamy, mixed, superactive, frigid).

Seeds of PSR23 cuphea were obtained from Oregon State University in 1998 and increased yearly at the Swan Lake Research Farm. At each of the seven farms in both 2002 and 2003 seeds of PSR23 cuphea, which were produced the previous year at Swan Lake, were sown at a rate of 1000 seeds m^{-2} in rows separated by 61 cm. Sowing depth was 1 cm. A roller-type packing implement was used to ensure good seed-soil contact after sowing. Immediately before sowing, soils were rototilled; fertilized with the equivalent of 112, 13, 30, and 52 kg ha^{-1} of N, P, K, and S, respectively; sprayed with the equivalent of 0.8 kg ai ha^{-1} of ethalfluralin [*N*-ethyl-

Table 1. Locations (nearest city, state, latitude, and longitude), daylength (h) on 21 June, planting dates (PD), and harvesting dates (HD) for seven experimental sites at which 'PSR23' cuphea was grown in 2002 and 2003. Abbreviations for planting and harvesting dates, A, M, J, S, and O represent April, May, June, September, and October.

Location	Lewis, IA	Castana, IA	Calumet, IA	Lamberton, MN	Morris, MN	Crookston, MN	Roseau, MN
Latitude (°′)	41 31	42 07	42 94	44 23	45 59	47 77	48 85
Longitude (° ′)	95 08	95 91	95 55	95 26	95 91	96 61	95 76
Daylength (h)	15.0	15.1	15.2	15.3	15.5	15.8	16.0
				2002			
PD1	16 A	16 A	17 A	25 A	26 A	02 M	01 M
PD2	08 M	08 M	08 M	14 M	14 M	22 M	22 M
PD3	29 M	29 M	29 M	06 J	06 J	17 J	n/a
HD	16 O	16 O	21 O	15 O	09 O	07 O	n/a
				2003			
PD1	15 A	15 A	15 A	22 A	24 A	01 M	01 M
PD2	07 M	07 M	07 M	13 M	13 M	21 M	21 M
PD3	27 M	27 M	27 M	03 J	03 J	13 J	12 J
HD	02 O	01 O	30 S	30 S	26 S	25 S	24 S

α,α,α-trifluoro-*N*-(2-methylallyl)-2,6-dinitro-*p*-toluidine)] for weed control (Forcella et al., 2005); and harrowed. Experimental areas were hand-weeded subsequently as necessary. Previous crops varied among locations from small grains to grain legumes.

There were three sowing dates at each location. These dates varied by about 3 wk within a location. The first sowing dates represented the earliest times that soils could be rototilled and sown at each location and, consequently, southern locations were sown earlier than northern locations (Table 1). Each sowing date was replicated four times within a location using a randomized complete block experimental design. Plots were 3.1 m wide and 3.1 m long.

All plots within a location were harvested on the same day. All plants within a 1-m^2 section of each plot were clipped at ground level, placed in bags, air-dried, and threshed. Seeds were cleaned through sieves and air-stream separators. Seed yields were calculated on the basis of kilograms of seed per hectare.

The same two researchers performed all management and sampling operations at all sites. This helped to prevent occurrence of location-specific errors. In contrast, however, because the researchers were based at Morris and the experimental sites were separated by long distances (1050 km), frequent visits to each site were not possible for regular phenological monitoring. Consequently, useful phenological information, such as the date of first flowering, could not be recorded accurately.

In 2003 the experiments at Lewis, Castana, and Morris were duplicated under irrigated conditions immediately adjacent to the dryland sites. Irrigation was applied at the discretion of the farm manager at each site. Although the amounts of water applied through simple lawn spinklers were not recorded at Lewis and Castana, the frequency and amount of irrigation assured that the plants grew better than their dryland counterparts. At Morris, water was applied through a portable irrigator with a single high-pressure nozzle that delivered 105 mm of water during the course of the 2003 growing season.

Weather data were collected at the research farm at each location (Table 2). Growing season (April through September) cumulative rainfall and average air temperatures were calculated. Daily minimum and maximum air temperatures were used to calculate growing degree-days (base 10°C) from sowing date to harvest date, and average daily temperature for September, the presumed principal time period of seed-fill for all planting dates. Because PSR23 is indeterminate and prone to seed shattering, a more accurate determination of seed-fill periods was not possible for this wide-spread series of experiments.

Oil Analyses

Seed oil content was determined by pulsed NMR (Bruker Minispec pc120, Bruker Analytische Messtechnik, Karlsruhe, Germany)[1] with a 0.47 T permanent magnet maintained at 40°C and providing hydrogen nuclei with a resonance of 20 MHz. The instrument was calibrated and checked with standards of known oil contents. About 2 g of clean seed randomly subsampled from the seeds harvested from each plot were used for oil analysis. Oil levels were expressed as a percentage of air-dry seed weights.

Gas chromatography (GC) of fatty acid methyl esters (FAMEs) was performed with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector (FID) and an autosampler/injector. Analyses were conducted on a sp. 2380, 30 m × 0.25 mm i.d. (Supelco, Bellefonte, PA). Saturated C_8–C_{30} FAMEs provided standards for calculating equivalent chain length values, which were used to make FAME assignments.

SP 2380 analyses were conducted as follows: column flow 1.4 mL/min with helium head pressure of 138 kPa (20 psi); split ratio 50:1; septum purge of 4 mL/min; programmed ramp 120 to 135°C at 20°C/min, 135°C to 265°C at 7°C/min; injector and detector temperatures set at 250°C.

FAMEs were made directly from whole seeds by placing 30 cuphea seeds in a 14.8-mL (4-dram) vial. Potassium hydroxide/methanol solution (0.5 *M*, 4 mL) was added to the vial and the seeds ground for 20 s with a VirTishear Mechanical Homogenizer (VirTis Company Inc., Gardner, NY) fitted with a 10-mm diameter shaft. The vial was then sealed with an aluminum lined cap and placed in a heating block maintained at 60°C. After 1 h, the vial was removed from the heating block and 4 mL of 1.0 *M* sulfuric acid/methanol solution was added. The vial was resealed and placed back in the heating block. After 15 min, the vial was removed from the heating block, and 2 mL of saturated sodium chloride solution were added followed by 4 mL of hexane. The contents of the vial were mixed thoroughly, and then the layers were allowed to separate. A 0.25-mL aliquot was removed from the top hexane layer containing the methyl esters and diluted to 2 mL with hexane in a crimp cap GC vial. The sample was then injected (1 μL) on the GC using the conditions described above. All samples were run in duplicate. Standard curves of methyl caprate, methyl stearate and methyl oleate were used to confirm response factors for the GC FID, which matched those previously reported by Ackman (1993).

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[1] Mention of trade names and companies does not constitute endorsement by USDA-ARS.

Table 2. Growing season (April to September) weather characteristics for seven locations at which 'PSR23' cuphea was grown during 2002 and 2003. Locations are arranged from south (Lewis, IA) to north (Roseau, MN).

Year–city	Lewis	Castana	Calumet	Lamberton	Morris	Crookston	Roseau
			Cumulative rainfall (mm)				
2002	368	395	313	442	502	617	565
2003	398	420	502	425	539	426	412
			Average temperature (°C)				
2002	19.5	19.0	17.8	17.0	16.3	15.1	14.3
2003	18.5	18.0	17.0	16.6	16.5	15.7	14.9
			Rainfall:temperature ratio (mm °C^{-1})				
2002	18.9	20.8	17.6	26.0	30.8	40.9	39.5
2003	21.5	23.3	29.5	25.6	32.7	27.1	27.7
			Average temperature (°C) in September (seed-fill)				
2002	19.5	18.4	17.3	16.9	17.2	15.7	14.9
2003	16.7	15.7	15.0	15.4	15.1	13.7	14.5

Statistical Analyses

Analyses of variance were calculated for most data with location as the main treatment and planting date as a subtreatment. Although percentage data for oil contents may require transformation before statistical analyses because of unequal variances among treatments, the variances of percentage values for seed oil and fatty acids usually were homogenous across sites (Bartlett's test, $p > 0.05$), and therefore, they were not transformed. The only exceptions were a few of the minor fatty acids and total oil in 2002. Arcsine, square root, and logarithmic transformation did not return equal variances for these variables ($p < 0.05$), and consequently, no transformations were used for final statistical analyses. Because of year × location interactions for several variables, ANOVA results are presented separately for each year. Linear regression also was used to explore relationships among seed yields, total oil, specific fatty acids, and weather variables. Statistical analyses were performed by Statistix 8 software (Statistix 8, 2003).

RESULTS AND DISCUSSION

Seed Yields

Seed yields under dryland conditions varied appreciably ($p < 0.05$) across locations both years (Fig. 1). High yields were recorded at Morris both years and at Roseau in 2003. (Roseau plots during 2002 were destroyed by region-wide flooding.) In contrast, uniformly low yields were recorded at Lewis, Castana, and Crookston, whereas yields at Calumet and Lamberton were always intermediate. Although average growing season temperature decreased steadily as latitude increased (Tables 1 and 2), this variable was not related closely with seed yields, probably because of the effects of water stress and other variables.

Crop water stress was more intense in southwestern Iowa during both 2002 and 2003 than elsewhere because of lower rainfalls and higher air temperatures compared with other locations. Simple rainfall-to-temperature ratios (Table 2) provide a sense of the increased likelihood of water stress in Iowa than in Minnesota. However, these dry conditions only partly explained low seed yields of cuphea in this region. For example, in 2003 cuphea produced twice as much seed at Lewis and Castana under irrigation compared with dryland treatments (Fig. 2), but even with irrigation, these yields still were low compared with dryland sites farther north. Apparently, other factors besides water stress contributed to low seed yields in Iowa.

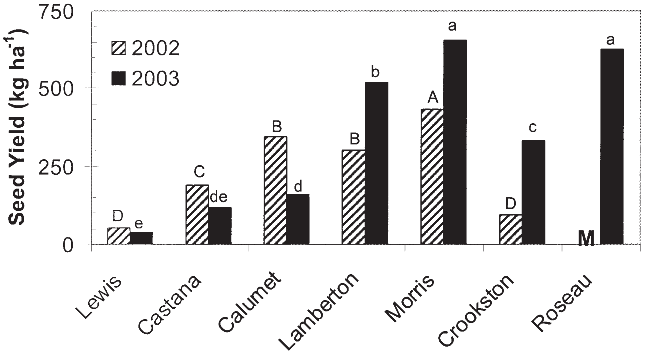

Fig. 1. Seed yields of dryland cuphea averaged across sowing dates for seven locations in Iowa and Minnesota during 2002 and 2003. Within years, differing letters indicate differences in seed yields ($p < 0.05$). Data for Roseau in 2002 are missing (M) because of regional flooding that destroyed all plots. Locations are arranged from south (Lewis) to north (Roseau).

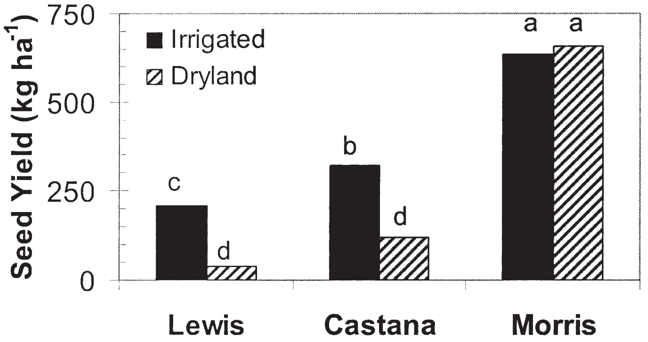

Fig. 2. Effect of irrigation on seed yield of cuphea at three sites during 2003. Differing letters atop columns indicate differences in seed yields ($p < 0.05$).

Aboveground dry weights were low at Iowa sites that were not irrigated and experienced drought stress, as would be expected. Some dryland sites and most irrigated sites in Iowa, however, produced cuphea dry weights comparable to those in Minnesota (Fig. 3). Thus, the lower seed yields in Iowa were not due entirely to smaller plant sizes resulting from drought or other adverse conditions. Instead, they somehow were related to the inability of PSR23 cuphea in Iowa to convert aboveground biomass into seeds.

Harvest indices were calculated by regressing seed yields against aboveground dry weights (Fig. 3) and comparing slopes for cuphea grown in Iowa and Minnesota. Harvest index for Iowa-grown cuphea (0.05) was lower ($p < 0.01$) than that for cuphea grown in Minnesota (0.14). Irrigation in Iowa increased aboveground dry weight but did not affect harvest index. Thus, even at Iowa sites that generated high aboveground dry weights of cuphea (e.g., 6000–8000 kg ha^{-1}), seed yields were nearly three times smaller in Iowa than in Minnesota (Fig. 3).

Planting date had inconsistent effects on seed yields under dryland conditions. Averaged across locations there was no effect ($p > 0.05$) of planting date in 2002, whereas such an effect did occur in 2003 (data not shown). At the three sites where irrigation was varied, apparent water stress interacted with planting date ($p < 0.05$) and enhanced its effect. For instance, in 2003 irrigated treatments showed no effects of planting date, but dryland treatments were affected ($p < 0.05$) by planting date (Fig. 4), with lower yields resulting from late planting, similar to the trends reported by Gesch et al. (2002b).

To summarize the yield results, first, the ability to produce seeds appears to rise with increasing latitude so that yields tend to be greater in Minnesota than in Iowa. Results from Crookston, MN, contradict this generalization: seed yields and harvest indices at this location were low to intermediate each year and, as yet, we cannot explain this phenomenon. Neither soil physical features, such as soil type (Table 1), nor weather conditions (Ta-

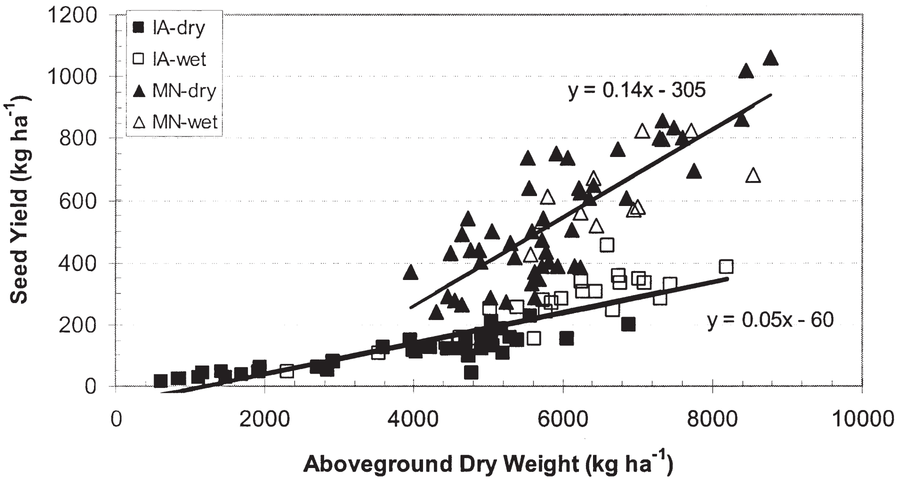

Fig. 3. Harvest index is the ratio of seed yield to aboveground dry weight, and was calculated from regression equations (slopes) to be 0.05 for cuphea grown in Iowa (IA), and 0.14 for cuphea grown in Minnesota (MN). Extremely low dry weights (<3000 kg ha^{-1}) and yields in Iowa were due to drought conditions. Closed symbols represent dryland plants (dry), and open symbols indicate irrigated plants (wet).

ble 2) at Crookston appeared anomalous. Second, early sowing dates enhanced seed yields under dryland conditions (Gesch et al., 2002b), but irrigation appeared to compensate, at least partially, for the yield penalty associated with late sowing.

Several explanations are possible for the observed trend of seed yield with latitude, and some of these will be discussed briefly in the following paragraphs. For instance, in growth chambers, PSR23 cuphea grows better under cool to moderate, than warm conditions. Gesch et al. (2002a) found that reproductive development of PSR23 follows a quadratic response to temperature, with the maximum being at about 23°C (average daily temperature). Reproductive development was reduced by more than 80% at temperatures of 15 and 31°C. Photosynthesis and dry matter accumulation followed similar quadratic responses, whereas water use efficiency was high only at low temperatures. Thus, higher summer temperatures at locations in Iowa may have inhibited growth and reproductive development relative to locations in Minnesota.

Cuphea in Iowa not only was planted earlier but also was harvested later than cuphea in Minnesota. Consequently, cuphea in Iowa potentially had a longer duration of seed shatter than cuphea in Minnesota. If this explanation has merit, then early planting dates should have had more shattering losses and lower yields than late planting dates, especially in the southern sites. This hypothesis was examined by plotting seed yields against growing season duration, which was measured in GDD or calendar days from sowing to harvest. Growing season duration did not explain differences among yields within or across sites (data not shown). There were no consistent trends in yields for early- vs. late-planted cuphea, and differences among sites were considerably greater than differences among planting dates.

Gesch et al. (2005) suggested that seed shattering may increase as harvest is delayed after the first killing frost in autumn. This suggestion also was examined using both calendar days and GDD after the first recorded minimum daily temperature of 0°C to explain low yields at some sites. Neither variable helped to explain yield differences among the locations (data not shown). Direct effects of other environmental variables, such as humidity, wind, diurnal temperature fluctuations, etc., on seed shattering of cuphea never have been studied.

Preliminary growth chamber experiments suggest that PSR23 cuphea is a facultative short day plant (data not shown). In other words, PSR23 can flower under several daylengths, but it flowers more quickly under daylengths <16 h. If true, then cuphea may flower earlier in Iowa than in Minnesota because of the former state's shorter daylengths before anthesis (Table 1). Earlier flowering corresponds to earlier seed set, and thus, the potential duration of seed shattering of cuphea sown in Iowa may have been significantly longer than that in Minnesota. Although this scenario is a possible explanation for low cuphea yields in Iowa, no direct evidence exists to support it.

Differential availability of insect pollinators, especially bumblebees (*Bombus* spp.), also could be a contributing factor to yield variability among sites. PSR23 is a facultatively cross-pollinated plant. A simple field experiment was performed in 2004 at the Swan Lake Research

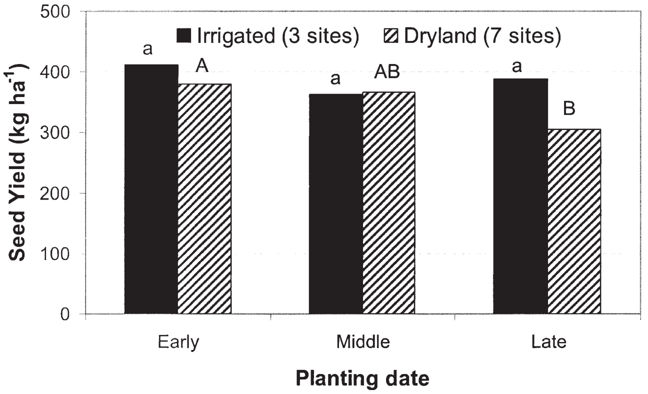

Fig. 4. Effect of planting date on seed yield of irrigated and dryland cuphea during 2003. Within each watering regime, letters atop columns that are not in common indicate differences in seed yields ($p < 0.05$).

Farm wherein 20 preanthesis flowers were tagged and left exposed to insect pollinators and another 20 preanthesis flowers were selfed by enclosure in perforated, translucent, insect-exclusion bags for 1 wk. Open-pollinated flowers produced 11.1 (±1.9) seeds fruit^{-1}, whereas self-pollinated flowers produced 2.6 (±3.7) seeds fruit^{-1}. Thus, because selfing within PSR23 apparently is only about a quarter as effective as outcrossing, seed yields conceivably could have been limited by inadequate cross-pollination at sites with naturally low populations of pollinators or weather-induced scarcity of pollinators, but no information exists to confirm this possibility. Most experimental sites were situated among other crop experiments or bulk crops, and they were not near woodlands, prairies, or pastures that might differentially harbor pollinators. However, the lowest yielding site, Lewis, IA, was adjacent to the research farm's horticultural garden; and the highest-yielding site, Morris, MN, was within 100 m of a lakeshore with a 5 m wide fringe of trees and shrubs.

Seed Oil

Oil content ranged from 27 to 33% of seed dry weight when averaged across planting dates (Table 3). This range of values is slightly greater than those reported previously for PSR23 (Gesch et al., 2002b, 2003, 2005; Knapp and Crane, 2000a), and slightly smaller than those for various genetic lines of the parental species, *C. lanceolata* and *C. viscosissima* (Knapp and Crane, 2000b). In 2002, highest oil percentages were found at the southern- and northern-most locations: Lewis and Crookston. In contrast, in 2003 seed oil levels were highest in northern sites and lowest in southern sites, and appeared to be associated inversely with average daily air temperature during September, the presumed period of seed-fill (cf., Tables 2 and 3). In contrast, levels of seed oil of soybean and other oilseed species typically show increasing or quadratic responses to air temperature during seed-fill (Piper and Boote, 1999). Possibly our oil and temperature data sets represent the down-trending section of a quadratic response of seed oil to increasing temperature. Perhaps the expected quadratic response would be apparent if PSR23 cuphea also had been grown in more northerly and colder environments, such as Manitoba, Canada, in addition to the sites in Minnesota and Iowa.

Oil percentage generally increased with higher seed yields (Table 3 and Fig. 1), with the exception of the high oil values and low yields at Lewis, IA, in 2002. Accordingly, in dryland plots, oil contents and seed yields were not related in 2002 ($r^2 = 0.04, p > 0.1$) and correlated poorly in 2003 ($r^2 = 0.35, p < 0.05$). However, irrigation in 2003 seemingly enhanced the relationship ($r^2 = 0.53, p < 0.05$).

Sowing date had slight but significant effects on oil percentages. Averaged across locations, early, middle, and late sowing dates had oil percentages of 28.5, 30.2, and 29.8% ($\mathrm{LSD}_{0.05} = 1.3$) in 2002 and 31.2, 31.2, and 30.1% ($\mathrm{LSD}_{0.05} = 0.5$) in 2003, respectively. Thus, the middle sowing dates resulted in oil values that were amongst the highest percentages across treatments both years. The overall lower values in 2002 than 2003 corresponded to the higher temperatures during seed-fill in 2002 compared with 2003 (Table 2).

To summarize the results for total seed oil content, average percentage seed oil was about 30% of dry seed weight. No location had seed oil percentages that were consistently higher than other locations along the transect. Generally, however, seed oil percentage was higher at more northerly sites, increased slightly with seed yield, and tended to be higher with middle sowing dates (early to mid May).

Fatty Acids

With the exceptions of capric and linoleic acids, most fatty acid concentrations were higher in PSR23 cuphea

Table 3. Seed oil contents (% seed dry weight) and fatty acid contents (% total oil) of PSR-23 cuphea seeds from plants grown in seven locations along a transect from southwestern Iowa to northwestern Minnesota in 2002 and 2003. Ratios below fatty acids abbreviations indicate the number of carbon atoms and double bonds in each fatty acid molecule.

Year and location	Total oil	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic
		8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
		%							
					2002				
Lewis	$\underline{31.7}$	$\underline{0.59}$	67.4	3.1	$\underline{5.2}$	$\underline{6.7}$	0.76	9.2	5.5
Castana	28.4	0.56	68.0	3.0	4.6	6.5	$\underline{0.79}$	$\underline{9.4}$	5.5
Calumet	27.5	0.51	68.0	2.9	4.4	6.2	0.72	8.7	6.5
Lamberton	28.8	0.56	68.4	3.0	4.8	6.3	0.71	8.7	5.8
Morris	29.2	0.52	67.2	3.0	4.8	6.4	0.76	9.3	6.1
Crookston	31.4	0.50	$\underline{69.8}$	2.9	3.9	5.7	0.67	8.1	$\underline{6.8}$
Roseau	na	na	na	na	na	na	na	na	na
$\mathrm{LSD}_{0.05}$	1.8	0.03	1.7	NS	0.7	0.4	0.05	0.5	0.5
					2003				
Lewis	28.4	$\underline{0.66}$	68.5	$\underline{3.2}$	$\underline{5.1}$	$\underline{6.7}$	$\underline{0.80}$	$\underline{9.1}$	4.6
Castana	29.4	0.63	69.8	3.1	4.6	6.3	0.75	8.9	4.9
Calumet	30.1	0.63	70.9	3.0	4.6	6.0	0.72	8.5	4.7
Lamberton	32.4	0.61	71.4	2.9	4.2	5.8	0.69	8.4	4.9
Morris	33.2	0.62	72.4	2.9	4.0	5.6	0.66	8.2	4.8
Crookston	32.4	0.57	72.5	2.9	3.9	5.4	0.62	7.6	5.6
Roseau	30.3	0.53	$\underline{73.2}$	2.7	3.4	5.1	0.63	7.1	$\underline{6.2}$
$\mathrm{LSD}_{0.05}$	0.7	0.02	1.2	0.1	0.5	0.3	0.03	0.2	0.2

Underscored values represent highest maxima within columns if significantly different ($p < 0.05$) from minima; NS means not significant and na means not available. Locations are arranged from south (Lewis) to north (Roseau).

from the southern-most sites (Lewis or Castana, IA) compared with other locations (Table 3). The predominant triglyceride was capric acid. It averaged about 68% of seed oil in 2002 and 72% in 2003. Capric acid concentrations have been reported to be as high as 83% for *C. lanceolata* and 76% for *C. viscosissima* (Graham and Knapp, 1989), but many of the genetic lines used in breeding programs have capric acid values similar to those of PSR23 (Knapp et al., 1997; Tagliani et al., 1995).

Capric and linoleic acid percentages always were higher in the northern-most than the southern-most locations. The results for linoleic and oleic acids, which were inversely related to one another, are consistent with reports for other oilseed species in that the lower temperatures during seed-fill of northern sites (Table 2) were associated with relatively high levels of linoleic acid and low levels of oleic acid (Kane et al., 1997; Sarmiento et al., 1998; Thomas et al., 2003; Wilcox and Cavins 1992; Yaniv et al., 1989).

Our results also showed a strong negative linear relationship between capric acid percentage and average daily air temperature during September (T_{Sep}), i.e., the presumed period of seed-fill. Linear regression ($n = 13$, 2002, and 2003 data combined; $r^2 = 0.79$; $p < 0.001$) resulted in an equation of the following form: oil % = $88.4 - 1.14 \times T_{Sep}$. We are not aware of any prior reports of capric acid levels being influenced by air temperatures during seed-fill. With regard to the other fatty acids, myristic, palmitic, stearic, and oleic acids were positively and linearly associated with T_{Sep} ($p < 0.01$), but caprylic, lauric, and linoleic acids were not related linearly to T_{Sep} ($p > 0.05$).

Sowing date had no effect on capric acid in dryland cuphea either year. However, under irrigation, capric acid levels in cuphea responded to both planting date and the extra water ($p < 0.05$). Averaged across watering regimes, capric acid was slightly higher with late sowing (71.8%) than early or middle sowing dates (70.9 and 70.7%; $LSD_{0.05} = 0.9$) perhaps because seed-fill of well-watered plants occurred later and under lower temperatures with late sowing. Averaged across sowing dates, seed oil from irrigated cuphea contained 72.0% capric acid compared with 70.2% for dryland cuphea ($LSD_{0.05} = 0.7$). There was an irrigation × location interaction: in other words, irrigation enhanced capric acid levels in the most northern irrigated site (Morris) but not at the southern sites (Lewis and Castana).

To summarize fatty acid results, capric acid was the primary fatty acid, and its concentration was higher in northern than southern sites. Sowing date had little effect on capric acid levels. Capric acid concentrations increased with irrigation, but only in more northern than southern locations. All other fatty acids were of minor importance and, except for linoleic acid, they had higher concentrations in southern than northern locations.

CONCLUSIONS

A potential new source of MCFA, PSR23 cuphea, grows well vegetatively across a broad latitudinal transect of at least 41 to 49° N, which includes the U.S. states of Iowa and Minnesota. However, for reasons unclear at this time, harvest index and seed yields are low for plants grown below 44° N. Between 44° and 49° N (i.e., Minnesota), and along a longitude of about 96° W, cuphea appears well-adapted and high-yielding. Oil percentages of seeds vary from about 27 to 33%, may increase with increasing latitude, and tend to be highest when the crop is sown in late April to early May. Capric acid constitutes from 67 to 73% of seed oil, and levels are higher in northern than southern growing regions. Thus, the environment at high latitudes apparently is conducive to high seed yields, possibly high levels of seed oil, and clearly high concentrations of capric acid in PSR23 cuphea. As domestication of this crop continues and improves, cuphea from northern farming regions of the USA, and cold-temperate regions elsewhere, may serve as an alternative source of MCFA, especially capric acid, to industry's current sole dependence on tropical palms.

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