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Seed Yield and Oil Content of Cuphea as Affected by Harvest Date

Russ W. Gesch,* Steven C. Cermak, Terry A. Isbell, and Frank Forcella

ABSTRACT

Cuphea (*Cuphea viscosissima* Jacq. × *C. lanceolata* W.T. Aiton) can serve as an oilseed crop substitute for small- and medium-chain triglycerides, which are in high demand for chemical manufacturing. Domesticated genotypes of cuphea show good potential for agricultural production, but their indeterminate growth may result in seed shatter if left in the field too long. Little information exists on when to harvest cuphea to obtain greatest seed yield and oil content. A study was conducted on a Barnes soil in west-central Minnesota to determine the best time to harvest cuphea when sown at an optimum time in the spring. Harvests were taken at 1- to 2-wk intervals from mid-August through mid-October during 2001 and 2002. Seed yields were greatest within a time period of about 20 d in late September to early October. Soon after a killing frost (≤−2°C), 5 October in 2001 and 9 October in 2002, yield declined sharply at a rate of about 10.6 kg ha^{-1} d^{-1}, probably due to increased shattering. However, shattering due to mechanical harvesting was greater than that from natural causes. Total seed oil content also was influenced by harvest date. Across years, oil content averaged 247 g kg^{-1} in August, increasing to 304 g kg^{-1} by late September and thereafter. For greatest seed yield and oil content, the optimum time to harvest cuphea is in late September to early October in west-central Minnesota. However, until more shatter-resistant, determinate genotypes are developed, improved harvest management is needed to reduce shatter-induced yield loss.

CUPHEA spp. (Lythraceae) seeds are rich in small- and medium-chain triglycerides (Graham et al., 1981). Although most species are tropical, some species thrive in temperate environments (Graham, 1989). Presently, the USA and other developed nations import several billion kilograms of coconut (*Cocos nucifera* L.) and palm kernel oil (*Elaeis guineensis* Jacq.) annually (FAO, 2003) to meet chemical manufacturing demands for medium-chain triglycerides used in making soaps and detergents, personal-care products, nutritional and dietetic products, lubricants, and related products (Thompson, 1984). Domesticated cuphea could serve as a substitute for these present sources of small- and medium-chain triglycerides. Recently, newly developed uses for vegetable oils show that cuphea could also serve as a replacement for petroleum-based products, thus further increasing its marketability. For instance, saturated estolides derived from medium-chain triglycerides have been shown to have physical properties comparable to, or in some cases exceeding those of, commercially available engine lubricants (Cermak and Isbell, 2002, 2004). Also, a mutant strain of *Cuphea viscosissima* Jacq., VS-320, has potential as a diesel fuel substitute, without requiring methyl esterfication (Geller et al., 1999).

Until recently, the primary barriers to commercial production of *Cuphea* spp. have been seed shattering, seed dormancy, and self-incompatibility (Hirsinger and Knowles, 1984; Knapp, 1990). However, through the interspecific hybridization of *C. viscosissima* and *C. lanceolata* W.T. Aiton, genotypes have been developed that are self-compatible, nondormant, and partially shatter resistant (Knapp, 1993). One such genotype that shows good agronomic potential is cuphea PSR23 (Gesch et al., 2002, 2003), a summer annual with an indeterminate growth habit (Knapp and Crane, 2000). However, PSR23 is still prone to shattering. When seeded in early spring in west-central Minnesota, domesticated cuphea PSR23 typically begins flowering in mid- to late July, and most of its reproductive growth occurs throughout August (Gesch et al., 2002). Often by mid-August in Minnesota, the first cuphea seed capsules to mature begin to split at their dorsal surface, leading to shattering (Gesch et al., 2002).

Because of the indeterminate growth and flowering of *Cuphea* spp., seed maturity on a single plant can vary considerably (Thompson and Kleiman, 1988), primarily along its vertical axis. This is also true of domesticated genotypes. For some wild *Cuphea* species, flowering can occur over a two- to three-month period (Hirsinger, 1985; Graham, 1989). Thompson and Kleiman (1988) separated seed of eight different *Cuphea* species collected at several locations into different maturity groups based on color ranging from green (least mature) to brownish-black (most mature) and assessed their oil content, fatty acid profile, and crude protein. Based on their criteria of maturity, they found that seed weight and protein, but not oil content, differed significantly. However, under field conditions, there are no known reports of whether cuphea oil content is affected by harvest maturity.

Obviously, if cuphea is to become a commercially successful new crop, much work is needed to determine when and how to harvest this indeterminate plant. The present study was designed to determine the best time to harvest cuphea to obtain maximum seed yield and oil content and the extent of seed shattering in the field.

MATERIALS AND METHODS

Plant Culture

The study was conducted in 2001 and 2002 at the Swan Lake Research Farm located 24 km northeast of Morris, MN (45°40′ N), on a Barnes soil (fine loamy, mixed, superactive, frigid Calcic Hapludoll). Cuphea (PSR23, *C. viscosissima* × *C. lanceolata* f. *silenoides*) (Knapp and Crane, 2000) was drill-

R.W. Gesch and F. Forcella, USDA-ARS, North Central Soil Conserv. Res. Lab., Morris, MN 56267; S.C. Cermak and T.A. Isbell, USDA-ARS, Natl. Cent. for Agric. Utilization Res., Peoria, IL 61604. Received 1 Sept. 2004. New Crops. *Corresponding author (gesch@morris.ars.usda.gov).

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Abbreviations: DOY, day of year; PM, physiological maturity.

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seeded at a rate of 6.7 kg ha^{-1} and an approximate depth of 0.01 m on 10 and 17 May in 2001 and 2002, respectively. Cuphea was sown on ground previously cropped with soybean [*Glycine max* (L.) Merr.]. The seedbed was chisel-plowed the previous fall and then harrowed just before planting. Fertilizer was incorporated into the top 0.15 m of soil before planting, at a rate of 112, 13, and 30 kg ha^{-1} N, P, and K, respectively. Nitrogen was added as urea (68.3 kg ha^{-1}) and diammonium phosphate (43.8 kg ha^{-1}), and K was added as potassium oxide. Immediately after planting, the seedbed was packed one time with a solid-stand seeder (model PS1572, Land Pride, Great Plains Manufacturing, Salina, KS). Plot size was 6.1 by 3.05 m in 2001 and 9.1 by 3.05 m in 2002, consisting of five rows spaced 0.61 m apart. Plots were replicated three times in a randomized complete block design. Monocot weed species were controlled chemically with sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio) propyl]-3-hydroxy-2-cyclohexen-1-one} (0.3 kg a.i. ha^{-1}) while dicot species were controlled by hand weeding until canopy closure. However, in 2002, because of an infestation by lambsquarters (*Chenopodium album* L.) and pigweed (*Amaranthus retroflexus* L.), all plots were treated with imazethapyr {2-[4, 5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} (0.032 kg a.i. ha^{-1}). In previous trials, cuphea was found to be relatively tolerant of imazethapyr at the rate used in this study (Amundson et al., 2003).

Harvest

Harvests were taken at 1- to 2-wk intervals from 13 August to 23 October in 2001 and from 16 August to 15 October in 2002. Cuphea was combine-harvested using a model 8-XP Massey Ferguson harvester equipped with a small-grain style head. The combine was adjusted so that the plants fed through it were threshed and all the seed, including a large amount of chaff, was collected. This was done to prevent seed loss once the plants were in the combine. In 2001, one center row 6.1 m long was sampled for yield while in 2002, three center rows 9.1 m long were harvested at each harvest date. In 2002, plant stand counts were made from 6 m of the center row, after harvesting, of the three harvested rows in each plot. No stand counts were made in 2001. The seed plus chaff was immediately spread thinly on a greenhouse floor and dried by forcing air over it with large fans. Any unbroken seed capsules were further threshed before screen cleaning all seed (model Vac-Away, Hance Corp., Westerville, OH). The seed was weighed and analyzed at a moisture content of approximately 50 g kg^{-1}. At three different harvest dates in 2002, plants were hand-harvested from separate plots to compare their seed yields with those taken by machine. For this purpose, 1 m of row was hand-harvested from the middle of a center row from three randomized plots at the three different harvest times. The plants were dried in a greenhouse before threshing, screen cleaning, and drying the seed for determining yield.

Seed Shattering

In both years, seed shattering in the field was measured by capturing seed in collection devices that were installed 25 July in plots randomly chosen for the final harvest in each year. Each seed collection grid consisted of an area of 0.61 m^2 where 12 plastic cups (surface area = 63.6 cm^2 per cup) were buried so that their tops were flush with the soil surface. The cup spacing was 0.15 by 0.33 m, allowing for two rows of three cups on each side of a meter row of cuphea. The collection grid was duplicated within each of three replicated field plots, and the duplicates were averaged for the final statistical analysis (total number of cups each year = 72). Each plastic cup had its bottom removed and replaced with a fine mesh screen to allow capture of seed and passage of rainwater. The seed collection devices were removed for analysis before the final machine harvest.

Seed Analysis

Cuphea seed oil content was determined by pulsed nuclear magnetic resonance (NMR) (Bruker Minispec pc120, Bruker Analytische Messtechnik, Karlsruhe, Germany) with a 0.47 T permanent magnet maintained at 40°C and providing H nuclei with a resonance of 20 MHz. The instrument was calibrated and checked with standards of known solid contents. Approximately 2 g of seed subsampled from the bulk seed of each plot was used for oil analysis. Total N and C were determined for 0.4-g subsamples of seed using a Leco CN-2000 combustion device (Leco Corp., St. Joseph, MI).

Regression techniques were used to examine the relationship between harvest date with seed yield, oil content, and seed characteristics. This was done using the REG procedure of SAS (SAS Inst., Cary, NC). For seed yield and oil content, both years were combined to capture some of the year-to-year seasonal variability into one equation. For other comparisons, ANOVA was performed using the GLM procedure of SAS, and least significant differences (LSD) at the $P = 0.05$ level were used to detect differences between means.

RESULTS

When cuphea was planted in mid-May, greatest seed yields were obtained when harvested in late September to early October in both 2001 and 2002 in west-central Minnesota (Fig. 1). The relationship of seed yield and harvest date was not found to significantly differ ($P = 0.05$) between years. Thus, the data from both years were pooled and used for regression analysis, revealing day of year (DOY) 276 as the estimated optimum harvest date, which was 146 and 139 d from the date of planting in 2001 and 2002, respectively. The estimated optimum harvest date corresponded closely with occurrence of the first killing frost (i.e., temperature ≤−2°C), which was DOY 278 (5 Oct.) and 282 (9 Oct.) in 2001 and 2002, respectively (Fig. 1). Mid- to late August seed

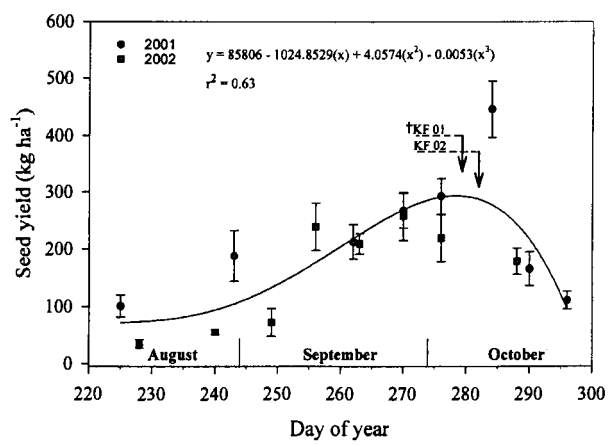

Fig. 1. Cuphea seed yield as affected by time of harvest in 2001 and 2002. The regression model and all parameter estimates were significant at $P \leq 0.05$. † Arrows indicate dates of first killing frost (KF; ≤−2°C) in each year; this was day of year 278 for 2001 and 282 for 2002. Values are means ± SE.

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yields were substantially less than those of late September to early October. During 2001, the average yield in August (144 kg ha^{-1}) was 68% less than the highest yield (446 kg ha^{-1}) recorded on 11 October (DOY 284) while in August 2002, mean yield of 45 kg ha^{-1} was 82% lower than the largest yield (258 kg ha^{-1}), which was obtained with harvest on 27 September (DOY 270). Using the regression of both years' data, seed yield increased at a rate of about 5.9 kg ha^{-1} d^{-1} between 1 September (DOY 244) and the estimated optimum harvest date 3 October (DOY 276). In contrast, between 7 and 23 October (DOY 280 and 296), yields sharply declined at a rate of about 10.6 kg ha^{-1}.

Between May and October of both growing seasons, mean monthly temperatures were similar but somewhat higher than the 116-yr average for data collected at a weather station within 19.3 km of the study site (Table 1). The similar growing season temperatures during 2001 and 2002 are reflected by the nearly equal number of accumulated growing degree days (°C d using a base temperature of 10°C; Table 1). Likewise, total precipitation received between May and October was very similar for both years and to that of the 116-yr average (Table 1).

Although a killing frost helped to dry plant material, making harvest easier, it also hastened seed shattering as evident by the sharp decline in seed yields that followed. The amount of seed shattered in the field was measured from near the beginning of flowering in late July until final harvest (Fig. 2). Seed shattered in the field during 2001 was only 11.4% of that of the highest yield recorded that season while it was 44% of the greatest yield in 2002 (Fig. 2). During the 2002 season, hand-harvested samples were taken on three different occasions to compare with those simultaneously mechanically harvested. Machine-harvested yields were significantly ($P < 0.01$) lower than those taken by hand except for the earliest harvest date tested (Table 2). On average, the ratio of machine- to hand-harvested yields was 0.43:1 (Table 2).

Seed oil content was influenced significantly ($P \leq 0.0001$) by harvest date in both years. The seed oil content as a function of harvest date fit a sigmoidal model (Fig. 3; $r^2 = 0.76$). Oil content was lowest in August, increased throughout September, and reached a plateau

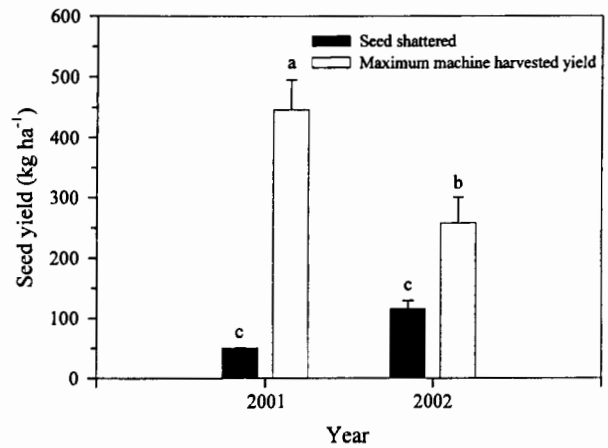

Fig. 2. Comparison of the amount of seed lost through capsule shatter in the field due to natural causes with maximum machine-harvested yield in 2001 and 2002. Cumulative seed shattered in the field was measured for the period of 25 July to when the final harvest was made for each year. Mean values followed by the same letter are not significantly different at the $P \leq 0.05$ level. Values are means ± SE.

in late September at about DOY 270 (Fig. 3). Average seed oil content during August was higher in 2001 (260 g kg^{-1}) than in 2002 (234 g kg^{-1}). However, at its peak in late September through October, average oil content was greater in 2002 (325 g kg^{-1}) than in 2001 (290 g kg^{-1}). In both years, seed oil content in August was significantly ($P < 0.05$) lower than it was in late September and October.

The 1000-seed weight significantly ($P < 0.001$) increased with harvest date in 2001 but not 2002 (Table 3). Seed N and C content were little affected by date of harvest (Table 3). The 1000-seed weight of seed obtained from the first harvest made in mid-August, DOY 225 and 228 for 2001 and 2002, respectively, was consistently lower than those for later dates. For both years, there did not appear to be any clear pattern for N or C content of seed by harvest date (Table 3).

In 2002, seed yield per plant dramatically increased, approximately 3.5-fold between early (DOY 249) and late (DOY 270) September and then declined thereafter (Fig. 4). Plant population, which was based on final stand counts, was not significantly different ($P \leq 0.05$)

Table 1. Monthly temperature, precipitation, and accumulated growing degree days (GDD) during the 2-yr cuphea harvest date study.

Month	Mean temperature 2001	Mean temperature 2002	Mean temperature 116-yr mean†	Total precipitation 2001	Total precipitation 2002	Total precipitation 116-yr mean‡	Total GDD§ 2001	Total GDD§ 2002
	°C			cm			°C d	
May	15.4	11.6	13.9	6.9	6.7	7.1	173	94
June	19.7	21.4	18.8	9.8	5.7	9.5	292	334
July	22.8	23.4	21.6	9.0	14.7	8.6	386	415
Aug.	21.7	20.3	20.1	5.4	8.6	8.3	361	319
Sept.	15.4	17.2	14.3	10.3	2.9	6.1	166	220
Oct.	8.0	3.6	7.7	3.0	6.7	5.5	28	11
Overall mean or total	17.2	16.3	16.1	44.5	45.3	44.9	1407	1393

† Calculated from daily mean temperatures collected between 1886 and 2002 at the West Central Research and Outreach Center weather station, University of Minnesota, Morris, MN.
‡ Calculated from daily precipitation collected between 1886 and 2002 at the West Central Research and Outreach Center weather station, University of Minnesota, Morris, MN.
§ Growing degree days (GDD) were calculated using a base temperature of 10°C.

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Table 2. Comparison of machine versus hand-harvested seed yield at three different harvest dates in 2002.

Harvest date	Accumulated GDD† from sowing to harvest	Machine-harvest yield‡	Hand-harvest yield	Mean yield by harvest date§	Ratio of machine to hand-harvest
DOY¶	°C d	kg ha^{-1}			relative
249	1206	72	158	115	0.46
263	1336	209**	484	346	0.43
276	1354	219***	541	380	0.40
Overall mean	1299	167	394	280	0.43

** Significant difference at the $P < 0.01$ level between machine- and hand-harvested yields at the respective harvest date. Comparisons were made using a factorial ANOVA with harvest date and method as main effects.
*** Significant difference at the $P < 0.001$ level between machine- and hand-harvested yields at the respective harvest date. Comparisons were made using a factorial ANOVA with harvest date and method as main effects.
† GDD, growing degree days.
‡ LSD (0.05) for harvest method = 91 kg ha^{-1}; the effect of harvest method on yield was significant at the $P < 0.001$ level.
§ LSD (0.05) for harvest date = 112 kg ha^{-1}; the effect of harvest date on yield was significant at the $P < 0.001$ level. The interaction between harvest method and harvest date was not significant.
¶ DOY, day of year.

across harvest dates for 2002, averaging 25 plants m^{-2} (data not shown).

DISCUSSION

Since cuphea has an indeterminate growth habit and little is known about its agricultural management, the primary objective of this study was to determine the best time for harvesting to achieve greatest seed yield and oil content. Trends in seed yield and oil content with respect to harvest date were similar across both years. This was probably caused by similar weather conditions during the growing season of both years. Results of seed yield modeled for harvest date indicate that the best time to harvest occurs between about DOY 265 and 285, with 276 being the estimated peak, if cuphea is planted in mid-May. For west-central Minnesota, early to mid-May was previously found to be the optimum time to plant cuphea (Gesch et al., 2002). For 2001, the optimum harvest time corresponded to a range of 135 to 155 d after planting while for 2002, it was 128 to 148 d.

Clearly, seed shattering was one of the reasons for the relatively short window of opportunity to obtain optimum yields of cuphea, and this was probably influenced by weather. Canola (*Brassica napus* L.) also experiences yield loss due to shattering if left in the field too long, and this problem tends to be exacerbated by unfavorable weather conditions (Elias and Copeland, 2001), which is probably true for cuphea as well. In this study, field observations suggest that desiccation of seed capsules, which was hastened by temperatures below −2°C, increased shattering. When averaged across three harvest dates in 2002, the amount of seed obtained by hand harvesting was 227 kg ha^{-1} greater than that obtained by mechanical harvesting (Table 2). The amount of seed collected from seed capsules shattering in the field (Fig. 2), averaged across 2 yr, was 83 kg ha^{-1}. These results indicate that seed loss due to mechanical harvesting may be greater than that caused naturally and thus suggests the need for harvesters designed for shatter-prone plants.

In both years of the study, seed yields were considerably lower than those previously reported for the same area (Gesch et al., 2002, 2003). Undoubtedly, part of the reason for the lower yields experienced in this study

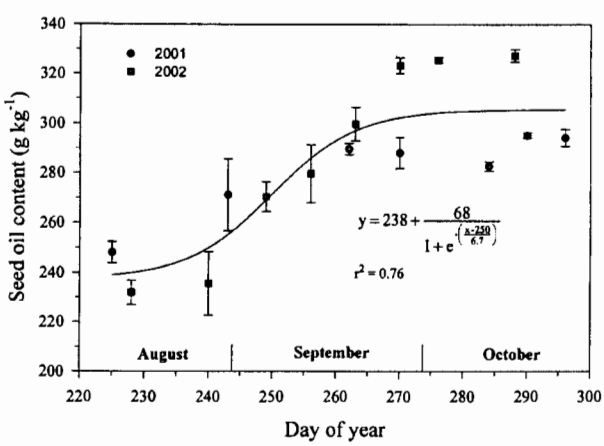

Fig. 3. Seed oil content as affected by time of harvest in 2001 and 2002. The regression model and all parameter estimates were significant at $P < 0.0001$. Values are means ± SE.

Table 3. Effects of harvest date on cuphea seed mass and N and C content.

Year	Harvest date	Accumulated GDD†	1000-seed wt.	N content	C content
	DOY‡	°C d	g	g kg^{-1}	
2001	225	961	2.9	32.3	542
	243	1153	3.1	33.4	560
	262	1280	3.1	34.4	561
	270	1302	3.2	32.8	555
	276	1334	3.2	34.4	559
	284	1342	3.2	32.1	543
	290	1346	3.2	33.8	566
	296	1346	3.4	33.4	559
$P > F$§			***	NS	NS
r^2			0.52	0.04	0.10
2002	228	972	2.8	32.7	528
	240	1093	3.8	31.1	474
	249	1206	3.6	36.5	530
	256	1284	3.5	35.5	534
	263	1336	3.5	34.6	547
	270	1339	3.5	33.9	552
	276	1354	3.4	34.2	555
	288	1363	3.5	32.7	556
$P > F$			NS	NS	**
r^2			0.10	0.01	0.38

** $P < 0.01$.
*** $P < 0.001$.
† GDD, growing degree days.
‡ DOY, day of year.
§ Probability that harvest date had a significant effect on the dependent variable in the regression model.

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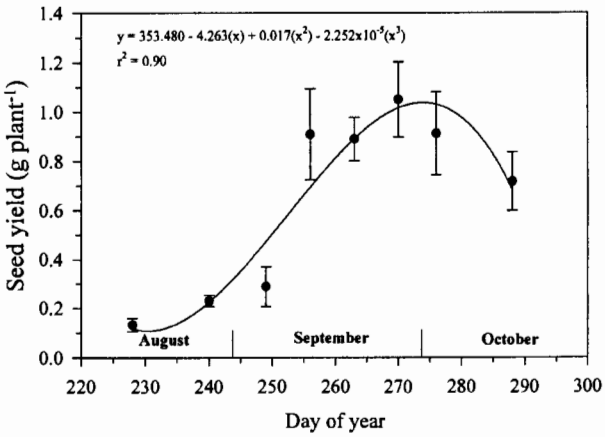

Fig. 4. Effect of harvest date on seed yield per plant during 2002. The regression model and all parameter estimates were significant at $P \leq 0.01$. Values are means ± SE.

was due to seed shatter caused by mechanical harvesting, whereas in previous studies, only hand harvesting was used. However, during the 2002 season, an appreciable weed infestation developed, and all plots were treated on 19 June (DOY 170) with imazethapyr to control the problem. Cuphea was temporarily stunted by the herbicide treatment, and the combination of weed pressure and herbicide treatment may be partially responsible for the poor yields in 2002.

Like yield, seed oil content was clearly influenced by harvest date. Oil content increased sharply from early to mid-September, stabilizing at about DOY 270 (Fig. 3). In contrast, seed weight, except in 2001, and N and C content were not clearly influenced by harvest date. Although, based on 1000-seed weights and N and C content of cuphea seeds, harvesting in August is probably too early for seed to reach physiological maturity (PM), at least in the region of this study. Typically, PM for many crops is determined to be the time at which seeds have reached their maximum dry weight (Elias and Copeland, 2001; TeKrony et al., 1979). However, for indeterminate species such as canola, there is evidence that PM, when based on seed quality, can occur several days after maximum dry matter accumulation (Still and Bradford, 1998). In the present study, cuphea appeared to reach maximum seed dry weight by early September (based on 1000-seed weights). However, because of the indeterminate nature of cuphea, additional seed was apparently filled during the early part of September as evident by a significant yield increase during this time, which at least in 2002, was not due to differences in plant population. In this study, the time at which seed obtained the highest level of oil content coincided well with that for optimum harvest yield.

Because cuphea continues to flower and set seed over a long period (Graham, 1989, and personal field observations), each harvest taken in our study probably contained a wide range in seed maturity. The time from flowering to maximum oil content of individual cuphea PSR23 seeds is not known. But, measurements of seed development in *Cuphea wrightii* and *C. lutea* indicate that maximum dry weight and oil content occur at about 19 to 21 d after anthesis (Kaliangile and Grabe, 1988). This is shorter than the time required for other oilseed crops such as canola, which when field-grown, requires about 32 to 36 d after flowering to reach maximum oil content (May and Hume, 1995). In our study, it is reasoned that the lower oil content of early harvested cuphea seed, especially that taken in August (average 247 g kg^{-1} across both years), resulted from a greater amount of immature seed. This is contrary to the findings of Thompson and Kleiman (1988), who found that oil content was little affected by seed maturity in eight different *Cuphea* species studied from nine geographical locations. It is important to note, however, that only wild *Cuphea* species were used in their study and that maturity was based on seed color rather than time after planting or anthesis.

This study is the first of its kind that we know of that was designed to determine the best time to harvest domesticated cuphea. Results showed that the best time to harvest cuphea to obtain greatest seed and oil yields in west-central Minnesota was late September to early October when sown at an optimum time. The window of opportunity to achieve greatest yields, however, is relatively short, primarily due to shattering. This study also reveals that seed development and improved harvest management to reduce yield loss due to shattering are important areas of further cuphea research. Cuphea genotypes with improved resistance to shattering and a more determinate growth habit may be necessary before large-scale commercial production is feasible.

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