Yield and Growth Response of *Cuphea* to Sowing Date

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ABSTRACT

Select germplasm of *Cuphea*, developed from an interspecific hybridization of *C. viscosissima* Jacq. and *C. lanceolata* f. *Silenoides* W.T. Aiton, shows good potential for commercial production in short-season temperate climates. *Cuphea* seed oil could serve as a domestic source of medium-chain fatty acids, which are in high demand by the chemical manufacturing industry. However, little is known about proper management practices for *Cuphea* agronomic production. A field study was conducted in west central Minnesota to determine optimum time of sowing in the northern Corn Belt region and describe influences of sowing date on growth and seed yield components of a semidomesticated germplasm line (PSR23). Seed was sown on 15 April, 1 May, 15 May, 1 June, and 15 June 1999 and 2000. Sowing in May resulted in greatest seed and seed-oil yields, which were as high as 1.09 and 0.29 Mg ha^{-1}, respectively. Seed yield declined as much as 31% when sown 15 April and 65% when sowing was delayed until 15 June. Plants developed from seed sown before June tended to form more branches and accumulated a greater amount of aboveground biomass. Typically, there was a distinct decrease in plant biomass accumulation and most seed yield components when sowing date was delayed until June. *Cuphea* PSR23 can be successfully grown in the northern Corn Belt, with early to mid May being the best time for sowing.

Since the 1960s, it has been recognized that several species from the genus *Cuphea* (family Lythraceae) produce seed uniquely rich in medium-chain fatty acids (MCFA) (Miller et al., 1964). Medium chain fatty acids such as caprylic (C8:0), capric (C10:0), lauric (C12:0), and myristic (C14:0) are in high demand by the chemical manufacturing industry for production of soaps and detergents, personal-care products, nutritional and dietetic products, lubricants, and related products (Thompson, 1984). Presently, MCFAs used for these purposes are derived primarily from coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guineensis* Jacq.) oils and petrochemicals (Thompson, 1984). In the USA, there are currently no crops grown that serve as an economical source of MCFAs.

Of about 260 species of *Cuphea* that have been identified to date, several are known to flourish in temperate climates (Graham, 1989). Previous work by others (Hirsinger and Knowles, 1984; Hirsinger, 1985) indicates that several *Cuphea* species exhibit favorable agronomic traits making them potential candidates for domestication. The largest barriers to prevent *Cuphea* from being produced commercially have been seed shattering, seed dormancy, and self-incompatibility, all of which are typical wild-type traits (Knapp, 1990). However, breakthroughs towards domesticating *Cuphea* as a commercial source of MCFAs have led to the development of germplasm lines that are self-compatible, partially nonshattering, and nondormant (Knapp, 1993a).

PSR23, a select line developed from an interspecific hybridization of *C. viscosissima* and *C. lanceolata* is a dicotyledonous, herbaceous, summer annual with an indeterminate growth habit, which shows good potential for field cultivation (Knapp and Crane, 2000). However, little is known about best agricultural management practices for its production. The objectives of the present study were to determine optimum time of sowing and effects of sowing date on growth and seed yield components of PSR23 in west central Minnesota.

MATERIALS AND METHODS

Plant Culture

The study was conducted in 1999 and 2000 at the Swan Lake Research Farm located 24 km NNE of Morris, MN (45° 40′ N) on an Sverdrup sandy loam soil (coarse-loamy, mixed, Udic Haploboroll). *Cuphea* (PSR23, *Cuphea viscosissima* Jacq. × *C. lanceolata* f. *Silenoides* W.T. Aiton) (Knapp and Crane, 2000) was sown by hand at a 1-cm depth and a rate of 1 g per m of row. Plots were constructed in a randomized complete block design with three replications. Each plot consisted of three 1-m rows spaced 0.25 m apart and oriented north-south. Before sowing each row, 1 g of seed (each seed weighs approx. 2.5 mg) was evenly mixed with a small portion of washed sand to facilitate equally distributing the seed within a 1-m row. The germination rate of the seed used for the study was found to be approximately 24% at 25°C. Because only a small quantity of *Cuphea* seed was available for the study, a row of soybean spaced 0.25 m on the east and west side of each plot was grown and pruned to the same height as *Cuphea* to reduce border effects. Plots were sown on 15 April, 1 May, 15 May, 1 June, and 15 June 1999 and 2000. Plots were manually watered periodically until plants emerged. In 1999, plots were fertilized with 80, 76, and 72 kg ha^{-1} of nitrogen, phosphorus, and potassium, respectively. The fertilizer was applied in soluble form and split into five applications at approximately weekly intervals. Application for each sowing date treatment began when plants first emerged. In 2000, 112, 13, and 30 kg ha^{-1} of N-P-K were incorporated at one time to all plots into the upper 0.2 m of soil before the first sowing date. All plots were hand-weeded until canopy closure.

Plant Sampling and Analysis

Across sowing date treatments, all three rows per plot were hand harvested at approximately 1000 GDD (°C d, with 10°C as the base temperature). In 1999, mean GDD at harvest across treatments was 996 ± 37 (SD) and in 2000 it was 969 ± 41. The criterion used for time of harvest of this indeterminate crop was based on observations of when the most mature seed pods began to dorsally split. The harvest dates in 1999 for the first through the fifth planting dates, in sequence, were 18 August, 24 August, 30 August, 7 September, and 5 October and in 2000 they were 16 August, 25 August, 1 September,

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Published in Crop Sci. 42:1959–1965 (2002).

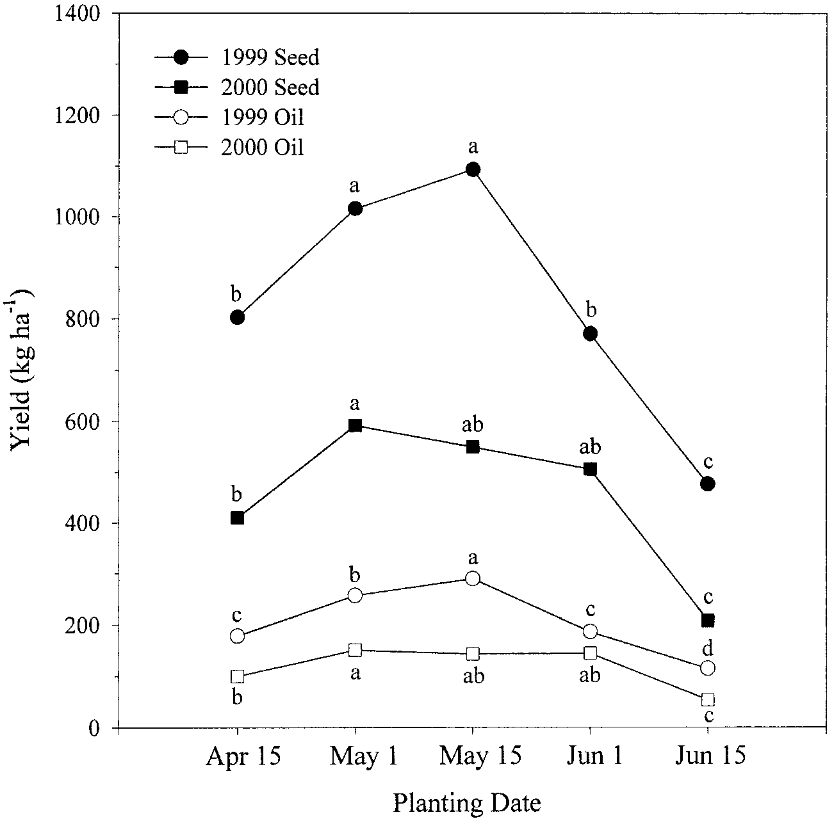

Fig. 1. Response of *Cuphea* seed and seed oil yield to planting date in 1999 and 2000. Within each year, mean values followed by the same letter are not significantly different at the $P = 0.1$ level.

14 September, and 26 September. After separating seed pods from plants, pods were air-dried in a greenhouse for 14 d, and then threshed and screen-cleaned by hand.

Immediately before harvest, three plants per plot were randomly sampled for analysis of growth and yield components. Plants for this analysis were clipped at the base of the stem, placed in wetted plastic bags, and transported back to the laboratory in a large cooler with ice. Leaf area was immediately measured with a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE). Branches were counted if they were ≥0.2 m. Dry weight of plant material was determined after drying in a forced air oven at 65°C for 60 h. Stand counts were taken at harvest. Additionally, plant height and initial flowering date were evaluated at weekly intervals throughout the study. Height from soil to the uppermost growing point was measured on plants in the center of each plot.

Cuphea seed oil content was determined by pulsed NMR (Bruker Minispec pc120, with an 18 mm absolute probe head, Bruker, The Woodlands, TX). The instrument was calibrated to oil extracted from bulk PSR23 *Cuphea* seed. Calibration samples were prepared by suspending known amounts of the oil on tissue to simulate seed oil. Approximately 2 g of seed subsampled from the bulk seed of each plot was used for oil analysis. Total nitrogen and carbon were determined for 0.4-g subsamples of seed with a Leco CN-2000 combustion device (Leco Corporation, St. Joseph, MI).

Data for each year were analyzed separately by the ANOVA procedure of SAS (SAS Institute, Cary, NC) with sowing date and replication as the main effects. Least significant differences (LSD) at the $P = 0.1$ level were used to detect differences between treatment means.

RESULTS

Sowing *Cuphea* in early to mid May resulted in highest seed yields in both years tested (Fig. 1). In 1999, the 15 May sowing date yielded an average of 1094 kg ha^{-1}, which was significantly greater ($P \leq 0.1$) than both the later two dates, and the earliest (15 April) date. Compared with the highest yield in each year, sowing as late as 15 June resulted in a 56 and 65% yield reduction in 1999 and 2000, respectively. Despite considerably higher yields in 1999 the trend of seed yield versus sowing date was similar in both years. The trend in oil yield was similar to seed yield (Fig. 1), mainly due to the relative stability of the seed oil content. In 1999, seed oil yield for the 15 May sowing date was significantly higher ($P \leq 0.1$) than those for the other dates and was as much as 2.5 fold greater than that for the latest sowing (Fig. 1).

Stand establishment was significantly affected by sowing date (Fig. 2). The plant population was similar in both years for the 15 April through 15 May sowing dates, averaging about 1.0×10^6 plants ha^{-1} in 1999 and 1.2×10^6 plants ha^{-1} in 2000. However, plant population significantly increased ($P \leq 0.1$) when *Cuphea* was sown 1 and 15 June (Fig. 2). In 2000, the population of established plants for the 1 June sowing date was nearly double that of the 15 May sowing date. Reduced plant stands for early sowing dates may have been largely due to colder soil throughout late April and May as compared with that in June (Fig. 3). The mean soil temperature at the 5-cm depth during May was 6.9 and

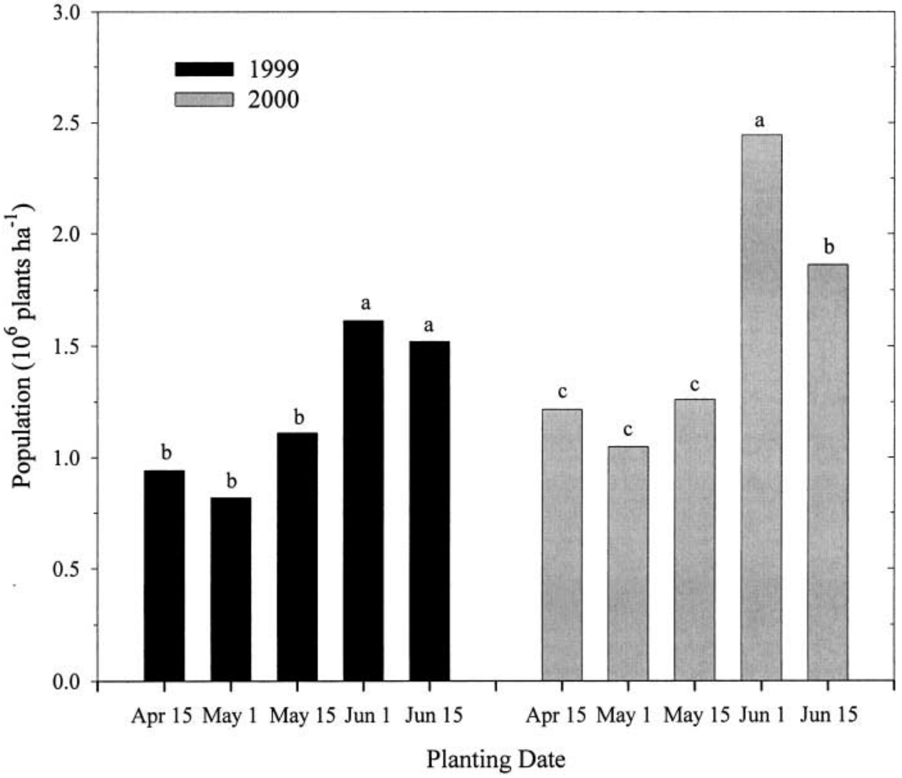

Fig. 2. Plant populations determined at harvest in 1999 and 2000. Within each year, mean values followed by the same letter are not significantly different at the $P = 0.1$ level.

4.4°C colder than that for June of 1999 and 2000, respectively. Seed sown in April and May remained in the soil considerably longer prior to emergence than the June sowing dates (Table 1), which may have caused poor germination and seed degradation.

As shown in Table 1, the number of days from emergence to flowering in 1999 ranged from 58 d for the 15 April sowing date to 46 d for the 15 June sowing, generally decreasing with later sowing date. The number of GDD units accumulated for each treatment between seeding and flowering, and emergence and flowering was relatively similar, ranging between 533 and 578°C d, and 445 and 500°C d, respectively (Table 1).

In 1999, the growth of plants prior to flowering, on the basis of height versus accumulated GDD units from the time of emergence, tended to be highest for the second sowing date, while the first and fifth dates were similar to each other and remained lower than the other

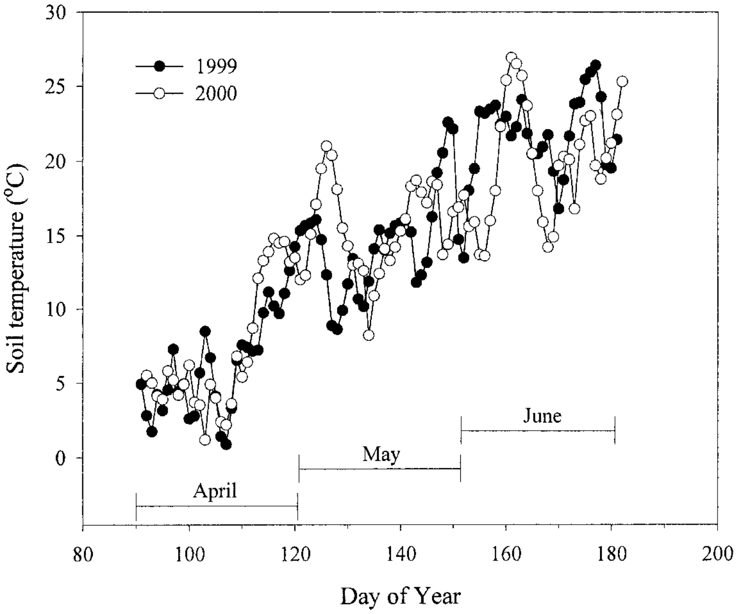

Fig. 3. Soil temperatures at the 0.05 m depth from April through June in 1999 and 2000.

Table 1. Effect of planting date on *Cuphea* initial emergence and flowering dates.

Date planted	Date of initial emergence†	Date of initial flowering‡	Days from emergence to flowering	GDD from seeding to flowering (°C d)	GDD from emergence to flowering (°C d)
15 April	15 May	12 July	58	559	489
1 May	24 May	12 July	49	533	445
15 May	27 May	17 July	51	548	483
1 June	9 June	26 July	47	564	471
15 June	24 June	9 August	46	578	500

† Initial emergence date was recorded as the date when seedlings were first observed on all three plots for a treatment with cotyledons fully above the soil surface.

‡ Initial flowering date was recorded as the date when flowers were first observed on at least one plant in all three plots for a treatment.

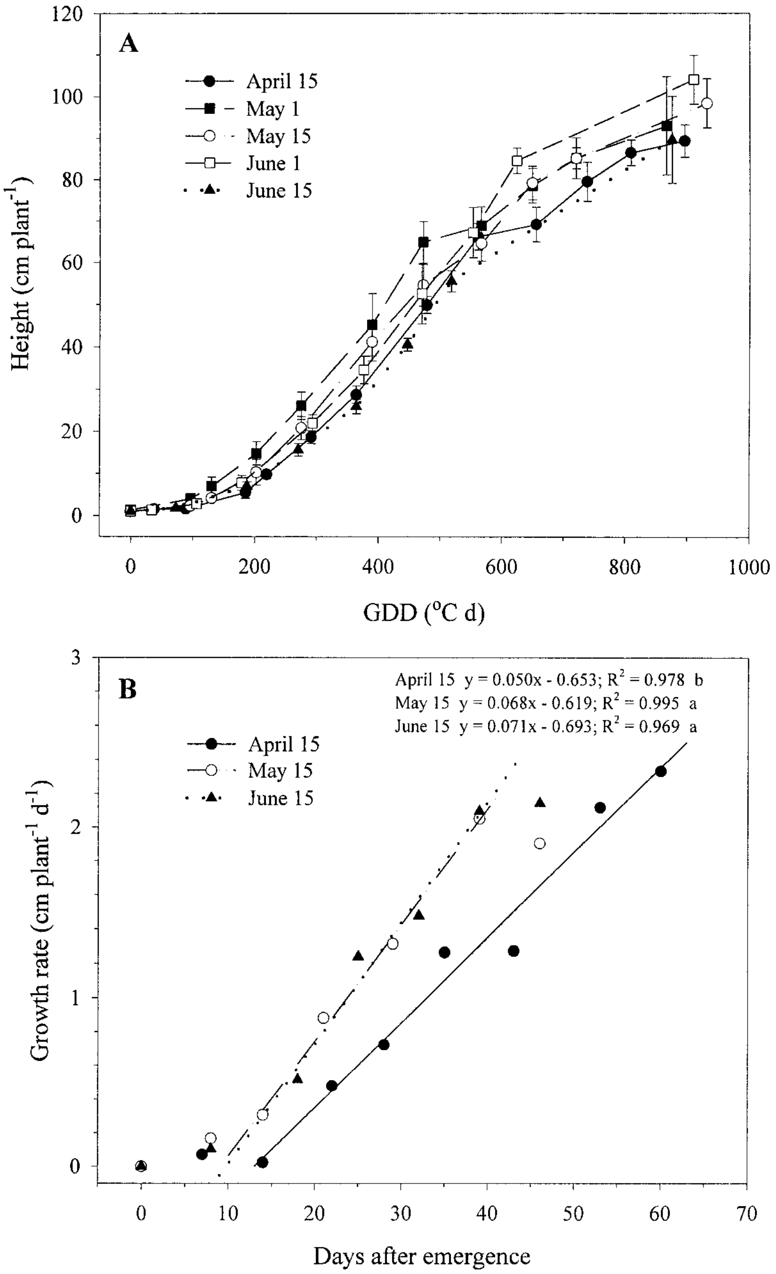

Fig. 4. (A) Height of *Cuphea* plants in 1999 as a function of growing degree days (°C d) accumulated from the time of emergence; values are the mean SD, $n = 3$. (B) Rate of height increase as a function of days after emergence. Regression lines are shown in B for data during linear growth phase; regression equations followed by a different letter are significantly different at the $P < 0.05$ level.

Table 2. Effect of planting date on growth characteristics of *Cuphea* sampled at time of harvest in 1999 and 2000.

Year	Planting date	Aboveground biomass	Height	Number of branches	Leaf area	LAI
		DW g plant^{-1}	cm plant^{-1}	plant^{-1}	dm^2 plant^{-1}	m^2 m^{-2}
1999	15 April	16.3a†	89.1c	11.8ab	12.8a	11.7a
	1 May	12.8ab	93.1bc	13.8a	12.4a	9.8a
	15 May	15.0a	98.5ab	13.1a	11.3a	12.2a
	1 June	8.7b	103.9a	9.4bc	6.8a	11.0a
	15 June	8.5b	91.7c	6.7c	NA‡	NA
	Means	12.3	95.3	11.0	10.8	11.2
2000	15 April	8.0a	96.0a	8.0a	9.0a	10.9a
	1 May	7.3a	97.9a	5.8ab	9.6a	10.0a
	15 May	7.1a	98.6a	4.3bc	9.3a	11.5a
	1 June	4.4b	90.0a	0.9d	4.4b	10.8a
	15 June	4.2b	67.0b	1.3cd	NA	NA
	Means	6.2	89.9	4.1	8.1	10.8

† Mean values within columns by year followed by the same letter were not significantly different at the $P = 0.1$ level.

‡ Not analyzed because of frost damage of leaves.

treatments throughout the experiment (Fig. 4A). Interestingly, at about 560 GDD, presumably during the time seeds were filling, the heights of plants across all treatments were almost identical. The growth rate of plants sown 15 April initially lagged behind that of the other four sowing dates (Fig. 4B; the second and fourth sowing dates were omitted for clarity but were similar in response to the third and fifth dates). This was expected since they were the earliest to emerge and soil (Fig. 3) and air temperatures (data not shown) were still quite low. On the basis of regression analysis of the linear phase of growth, the growth rate of plants sown 15 April was significantly lower ($P < 0.05$) than the other four treatments, which were not found to differ from each other (Fig. 4B).

Growth and Yield Components

Sowing date significantly influenced several growth and yield characteristics of plants. Generally, *Cuphea* sown before 1 June resulted in plants that accumulated a greater amount of aboveground dry matter by harvest than those planted 1 and 15 June (Table 2). This was likely due to branching, which also tended to be greater for the first three sowing dates (Table 2). There was no clear trend in the response of plant height to sowing date, except that the 15 June sowing consistently resulted in shorter plants. Leaf area index (LAI) values were quite large both years. Leaves form as opposite pairs on the stem and branches of *Cuphea* and begin forming only a few centimeters above the base of these organs. At the time plants were harvested, very few leaves even in the lower canopy senesced and excised, which is likely why LAI values were large. At harvest, LAI was relatively constant between the 15 April and 1 June sowing dates in both year. However, leaf area per plant was lower for the 1 June sowing, but only found to be significant in 2000 ($P = 0.1$; Table 2). Leaf area was not measured for plants of the latest sowing date due to frost damage. In both years, dense canopy closure between the 0.25-m rows occurred across all sowing date treatments by late July.

Because *Cuphea* population density increased with the 1 and 15 June sowing dates, total aboveground biomass accumulation may have been affected by plant competition. Aboveground biomass and number of branches per hectare were estimated by multiplying the mean values of these characteristics per plant, from Table 2, by population density (Table 3). On a land area basis, estimated aboveground biomass did not follow a clear trend in either year except that the 1 May sowing was lower than the 15 April planting date in both years (Table 3). The estimated number of branches per ha clearly declined with sowing date in 2000, but in 1999 it increased slightly between the 1 May and 1 June sowing dates before decreasing again with the 15 June sowing (Table 3).

Like dry matter accumulation and branching, the number of seed pods and seed weight produced per plant were generally not different across the 15 April to 15 May sowing dates, but were distinctly higher than the 1 and 15 June (Table 4). Seed size, based on 1000 count seed weights, was greatest for the 1 May and 15 May sowing date in 1999, and 15 May and 1 June in 2000, while size decreased with earlier and later sowing dates (Table 4). In 2000, the number of seeds per pod did not significantly differ across sowing dates (Table 4).

Total seed oil content, which was similar in magnitude between years, was affected by sowing date in 1999. Oil content was greatest for the 15 May sowing and was as much as 44 and 25 g kg^{-1} higher than 15 April and 15 June sown plants, respectively (Table 4). In 2000, the seed oil content was greatest for 1 June sown plants and not found to differ among the other four treatments,

Table 3. Effect of planting date on *Cuphea* biomass and branching on a land area basis.

Year	Planting date	Aboveground biomass	Number of branches
		DW Mg ha^{-1}	10^6 ha^{-1}
1999	15 April	15.4a†	11.1bc
	1 May	10.5b	11.3bc
	15 May	16.7a	14.6ab
	1 June	14.0ab	15.2a
	15 June	12.9ab	10.2c
2000	15 April	9.8ab	9.7a
	1 May	7.7c	6.1b
	15 May	9.0bc	5.4b
	1 June	10.8a	2.2c
	15 June	7.8c	2.4c

† Biomass and branching per area were calculated by means of per plant growth characteristics and the plant population density for the given sowing date. Mean values within columns by year followed by the same letter were not significantly different at the $P = 0.1$ level.

Table 4. Effect of planting date on seed yield components and total seed carbon, nitrogen, and oil content of *Cuphea* sampled at time of harvest in 1999 and 2000.

Year	Planting date	Number of pods	% Filled pods	Seeds per pod	Seed weight	1000-Seed weight	Carbon content	Nitrogen content	Seed oil content
		plant^{-1}	plant^{-1}	plant^{-1}	g plant^{-1}	g	g kg^{-1}	g kg^{-1}	g kg^{-1}
1999	15 April	120.7a†	78.6a	NA‡	1.23ab	2.50c	521b	26.8a	222c
	1 May	101.1ab	75.8a	NA	1.20ab	2.83a	525ab	28.0a	254ab
	15 May	117.3a	74.6ab	NA	1.50a	2.83a	535a	29.7a	266a
	1 June	63.6bc	64.5b	NA	0.69bc	2.67b	517a	26.6a	242bc
	15 June	53.6c	69.6ab	NA	0.38c	2.67b	522ab	28.7a	241bc
	Means	91.3	72.6		1.0	2.7	524	28.0	245
2000	15 April	47.1a	66.1a	13.0a	0.44a	2.62b	531a	32.7ab	243b
	1 May	44.7ab	69.1a	11.8a	0.43a	2.62b	532a	32.9a	255b
	15 May	47.9a	65.9a	11.4a	0.49a	2.77ab	532a	32.2bc	258b
	1 June	23.4bc	64.1a	10.8a	0.29ab	2.84a	538a	31.7c	284a
	15 June	13.2c	69.6a	11.3a	0.12b	2.60b	534a	30.3d	257b
	Means	32.3	67.0	11.7	0.35	2.69	533	32.0	259

† Mean values within columns by year followed by the same letter were not significantly different at the $P = 0.1$ level.

‡ Not analyzed.

although again the earliest sowing date tended to have low oil content (Table 4). Seed of *Cuphea* varieties developed from crossing *C. viscosissima* and *C. lanceolata* are particularly rich in capric acid (C:10) (Knapp, 1993b). A profile analysis of seed oil from the 1999 sowing date experiment revealed that capric acid (C:10) made up 70 to 75% of the total oil content with only small amounts of other fatty acids present (data not shown). The nitrogen content of seed on average was 14% greater in 2000 than in 1999, but carbon content was similar. Both nitrogen and carbon content differed little across sowing dates within years. However, nitrogen content did decrease from the 15 May to 15 June sowing dates in 2000 (Table 4).

DISCUSSION

In west central Minnesota where this study was conducted, optimum growth and yield of *Cuphea* (PSR23) was achieved by sowing in early to mid May. This seeding period is similar to that found optimum for production of early maturing soybean cultivars (i.e., maturity groups 00-I) grown in the northern Corn Belt region. Higher seed yields of *Cuphea* planted in May were largely due to a greater number of seed-pods and mass of seed produced per plant (Fig. 1 and Table 4). *Cuphea* plants produce seed pods on their branches and main stem (Graham, 1989). The higher number of pods per plant in the present study was primarily due to greater branching (Table 2). However, there was a relatively high degree of variability among individual plants, which may in part have been due to uneven plant densities, and *Cuphea*'s semidomesticated nature (Knapp and Crane, 2000). Also, border effects due to small plot size may have introduced variability. In both years of the study there was a distinct decline in most plant growth characteristics as well as seed yield when sowing was delayed until 1 June.

Sowing date significantly influenced plant stand establishment. Stand establishment for the 1 June and 15 June sowing dates was significantly greater than the earlier three dates (Fig. 2) and coincided with rising soil temperatures (Fig. 3). Low population densities for the earlier sowing dates were likely due to poorer germination and emergence resulting from cold soil temperatures throughout April and May (Fig. 3).

High plant densities can lead to greater interplant competition for available environmental resources such as light, soil moisture and nutrients (Adams, 1967). The higher stand density for the later two sowing dates might have contributed to the generally smaller and less branched nature of plants because of interplant competition. Soybean and dry bean (*Phaseolus vulgaris*) tend to have a similar growth habit to *Cuphea*. Parvez et al. (1989) working with soybean, and Bennett et al. (1977) studying dry bean, have shown that increasing plant population density significantly decreases branch and pod numbers per plant. Alternatively, in the present study, plants sown from 15 April to 15 May had a longer vegetative period, thus perhaps allowing them to form more branches prior to diverting plant resources into setting seed.

Increased population for the 1 and 15 June sowing dates, on a land area basis, compensated for the reduction of biomass per plant (Table 3). Additionally, population density for the later two sowing dates compensated for reduced branching per plant in 1999, but not in 2000 (Table 3). However, population density did not fully compensate for seed yield loss by reduced plant growth. When seed weight per plant values (Table 4) were used to calculate the mass of seed on an area basis, accounting for population, the yields were slightly higher but the trends similar to those shown in Fig. 1.

During the 1999 growing season, plant height increased with sowing date up to 1 June and then sharply declined with the 15 June sowing (Table 2). The response was not as clear in 2000 although plants seeded 15 June were much shorter than those of the other treatments, which could have been due to reduced late-season growth caused by unusually hot and dry conditions. Despite generally shorter plants for early-sown *Cuphea*, they consistently formed more branches and pods per plant than those planted in June. Sowing date effects on *Cuphea* morphology are somewhat similar to that of soybean. April sowing of both determinate (Beatty et al., 1982) and indeterminate (Akhter and Sneller, 1996) cultivars of soybean has been shown to promote more branches and pods per branch than sowing in June. Akhter and Sneller (1996) note that the branching response to sowing date is greater in indeterminate than determinate soybean cultivars. Also, Beatty et al. (1982) observed that height of soybean was greatest when planted in mid May and declined with earlier (mid April) and later (mid June) sowing dates. High plant population can also affect plant height, typically causing it to increase (Weber et al., 1966). In the present study, the height of *Cuphea* might have been affected by plant population density, although plants from the later sowing dates were occasionally shorter than those from earlier sowing dates (Table 2).

Seed oil is the primary product of importance for *Cuphea*. In our study, the total oil content of seed averaged 245 g kg^{-1} across all treatments in 1999, while in 2000 it was 259 g kg^{-1} (Table 4). The highest seed oil content found was for the 1 June sowing in 2000, which was 284 g kg^{-1}. This is slightly lower than that previously reported (Knapp and Crane, 2000). Seed oil yield per hectare mirrored seed yield (Fig. 1) because seed oil content was relatively stable across sowing dates. The best sowing date for total oil yield was not as clear in 2000, as differences across the 15 April to 1 June were smaller than in 1999 (Fig. 1). This could have been caused by poor growth conditions in late July through early September of 2000, which were hot and dry. Both vegetative and reproductive growth were substantially less in 2000 than they were during the 1999 growing season (Tables 2, 3, and 4). Late July through August may be a particularly critical period as this tended to be when most reproductive growth of *Cuphea* occurred (field observation). At a weather station located about 100-m from the test plots, the amount of precipitation recorded in August was 9.8 cm for 1999 as compared with only 2.9 cm in 2000 (data not shown). Alternatively, differences in seed and seed oil yield between years might have been due to N fertilizer management. Before the first sowing date in 2000, fertilizer was applied one time. This may have presented the opportunity for N to be lost to leaching and denitrification before later sowing dates.

Our data indicate that *Cuphea* can be successfully grown in the northern Corn Belt when seeded in the spring at about the same time that is optimum for early maturing soybean. Further agronomic and genetic research, however, is needed to optimize management protocols before *Cuphea* can be commercially produced.

ACKNOWLEDGMENTS

The authors thank Dr. Steven Knapp and Jimmie Crane for kindly providing the *Cuphea* germplasm for this study and their useful comments in constructing the study. We also acknowledge funding by Procter and Gamble and thank Joe Boots and Gary Amundson for expert field assistance.

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