Short communication

Differential sensitivity to temperature of cuphea vegetative and reproductive growth

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Abstract

Cuphea (*Cuphea viscosissima* Jacq. x *C. lanceolata* W.T. Aiton; PSR23) is a new oilseed crop rich in medium-chain fatty acids similar to tropical palms. Agronomic studies suggest that temperature is a key determinant of cuphea seed yields. However, little is known about the growth and photosynthesis response of cuphea to temperature. The following study is the first of its kind to evaluate cuphea's growth and photosynthesis response to temperature. Cuphea was grown under day/night temperature regimes of 18/12, 24/18, and 30/24 °C and regression analysis was used to assess its responses of growth and photosynthesis and determine their optimum temperature range. Vegetative growth and leaf photosynthesis adapted well over the temperature range studied. However, reproductive growth was more sensitive showing a decline with increasing temperature. Reproductive growth rate was greatest under the lowest (18/12 °C) temperature treatment and declined by 43% at the highest growth temperatures. In contrast, vegetative growth, which was greatest under the 24/18 °C treatment, declined by just 25 and 10% at the lowest and highest temperatures, respectively. Photosynthesis acclimated to temperature by up-regulation of *in vivo* Rubisco activity with declining growth temperature. Maximum Rubisco activity (V_{cmax}) in leaves under the 18/12 °C treatment was 76% greater than that of leaves grown at 30/24 °C. Photosynthetic acclimation permitted cuphea to vegetatively grow well over a wide temperature range, but does not explain the sensitivity of reproductive growth to temperature, which will require further research to elucidate.
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1. Introduction

Medium-chain fatty acids (MCFA) such as capric, lauric, and myristic acids are used extensively for manufacturing detergents, lubricants, personal-care products, and confectionaries (Thompson, 1984). Currently, the world's primary plant-derived sources of MCFA are coconut and oil palm (*Cocos nucifera* L. and *Elaeis guineensis* Jacq.). The genus *Cuphea* is also known as a rich source of MCFAs (Miller et al., 1964) and many species thrive in temperate climates (Hirsinger, 1985; Graham, 1989).

Through the interspecific hybridization of two North American natives, *Cuphea viscosissima* Jacq. and *C. lanceolata* W.T. Aiton, Knapp and Crane (2000) developed PSR23 cuphea, a germplasm line with seed retention, non-dormancy, and self-compatibility superior to its wild-type relatives. PSR23 has been the focus of most agronomic research (Forcella et al., 2005).

To better understand cuphea's growth and yield potential at different latitudes, Forcella et al. (2005) grew cuphea at several field sites between Iowa and Minnesota, transecting a latitudinal gradient of approximately 41 to 49°N. Vegetative growth of plants differed

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little among sites when drought was absent (some sites had irrigated and non-irrigated plots). However, seed yield dramatically differed and was significantly greater between approximately 44 and 49°N than more southerly latitudes. Although a myriad of biotic and abiotic factors may have affected their results, temperature was cited as a potential key factor (Forcella et al., 2005).

There is little published information about the growth and photosynthetic response of cuphea to temperature, although this information would be useful for understanding its adaptability as a new crop to different climates. Therefore, the objectives of the following study were to evaluate the responses of cuphea growth and photosynthesis to temperature to obtain knowledge of the optimum temperature range for growth and to determine its potential for photosynthetic acclimation over a range of daily growth temperatures.

2. Materials and methods

2.1. Plant material and growth conditions

Cuphea (PSR23) (Knapp and Crane, 2000) seeds obtained from first-generation plants grown in the field in west central MN, USA (45°40′N latitude; original seed came from S. Knapp, Corvallis, OR, USA) were pre-germinated and sown in 19 L pots filled with a 1:1:1 mixture of a Barnes soil (fine loamy, mixed, superactive, frigid Calcic Hapludoll), peat moss, and sand. For each temperature treatment, 14 pots containing three seedlings each were started in a greenhouse under a 28/20 °C day/night temperature with supplemental lighting to give a 16 h photoperiod. The 19 L pots were found to be sufficient size to not restrict cuphea root growth. At 6–7 days after planting (DAP) when all plants had emerged they were transferred to growth chambers (Environmental Growth Chambers, Chagrin Falls, OH, USA)[1] and grown under their respective treatment conditions for the remainder of the experiment. Treatments consisted of daytime maximum/nighttime minimum temperature regimes of 18/12, 24/18, and 30/24 °C giving daily mean temperatures of 15, 21, and 27 °C with a 16 h photoperiod. Two chambers were used in the study and after completion of the 18/12 and 24/18 °C treatments the 30/24 °C treatment was initiated. The treatments were carried out until 96–118 DAP.

The growth chambers were programmed to give modified diurnal sinusoidal temperature patterns that mimic natural diurnal temperature patterns. At the start of the photoperiod, 0600 central standard time (CST), temperature increased to a maximum by 1130 CST, remaining there until 1530 CST before declining gradually to the nighttime minimum by 0430 CST. Relative humidity was controlled at 55–65% during the day and 65–75% at night. Photosynthetically active radiation (PAR) was measured with a PAR-80 Linear PAR sensor (Decagon Devices, Pullman, WA, USA) and was maintained at approximately 550 μmol m^{-2} s^{-1} at plant height (light banks were adjustable and light levels were measured routinely on a weekly basis).

In each chamber, temperature and relative humidity were measured with two independent sets of sensors that were both shielded and aspirated. One of the sensors was integral to the chamber environment control system and data were continuously monitored and stored on a dedicated PC, while the other sensor, a HMP 45 (Vaisala, Woburn, MA, USA) was connected to a CR23X data logger (Campbell Scientific, Logan, UT, USA) for recording and storing data. Sensors were compared daily in both chambers and agreed well throughout the study. On average, measured temperatures varied ±0.2, 0.4, and 0.4 °C around the set points for the 18/12, 24/18, and 30/24 °C chambers, respectively, on a daily basis.

Plants were fertilized once per week with a commercial fertilizer (Peat-Lite Special, 20-19-18, N-P-K, Peters, Milpitas, CA, USA) and initially watered every 2–3 days. At 40 DAP all pots were integrated to an automatic watering system. Pots were rotated within a chamber weekly to minimize chamber microclimate effects.

2.2. Plant sampling

Plants were sampled approximately every two weeks beginning at 26 DAP. Three plants from each of two pots were sampled at each interval by cutting at the soil surface. Pots were numbered at the beginning of the experiment, and a randomized sampling plan was generated using the Plan procedure of SAS (SAS Institute, Cary, NC, USA) for sampling at each time interval. Individual plants were separated into leaves, stems and branches, and reproductive organs including buds, flowers, and capsules plus seed. Dry weights of plant material were recorded after drying in a forced air oven for 48 h at 65 °C.

Although cuphea (PSR23) has some degree of self-fertility (Forcella et al., 2005), it is a strongly insect cross-pollinated plant (Knapp and Crane, 2000).

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[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Because no pollinators were used in this study, reproductive organs (i.e., buds, flowers, and capsules plus seed) were pooled for each plant and treated as total reproductive biomass. Because the study was exploratory, and developmental differences due to growth temperature were anticipated, growth data were plotted as a function of DAP and regression was used to estimate growth rates for comparison and integrate variability inherent to any chamber differences. Between the second (40 DAP) and final samplings of all treatments, growth of plants progressed linearly and thus, growth rates were determined as the slope from the linear response phase. Growth rates were also plotted as a function of growth temperature to evaluate the response.

2.3. Photosynthesis measurements

Leaf photosynthesis was measured with an LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA) on newly, fully expanded leaves. At 40 DAP, leaf photosynthesis was measured on six individual leaves from different plants at midday under maximum growth temperature, a CO_2 concentration of $400\,\mu mol\,mol^{-1}$ (the approximate atmospheric level of CO_2 in chambers, which was measured periodically throughout the study), and $1200\,\mu mol\,m^{-2}\,s^{-1}$ PAR provided by the LI-6400 light source.

Photosynthesis as a function of internal CO_2 concentration (C_i) was determined within 2 h around midday at 48 DAP on three to four individual leaves from different plants for all treatments. Measurements were made for all treatments at a common leaf temperature of 24 °C and saturating PAR of $1200\,\mu mol\,m^{-2}\,s^{-1}$. Maximum *in vivo* Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) activity (V_{cmax}) and electron transport rate of RuBP (ribulose 1,5-bisphosphate) regeneration (J_{max}) were estimated from photosynthesis versus C_i data using Photosyn Assistant software (Dundee Scientific, Dundee, UK), which utilizes equations of Farquhar et al. (1980) to model photosynthesis versus C_i response.

2.4. Statistical analysis

Growth data were plotted as a function of DAP and linear regression used to determine slopes (i.e., growth rate) during the linear phase of growth, which were then analyzed for significant treatment effect using the GLM procedure of SAS (SAS Institute, Cary, NC, USA). Modeling the response of growth rates as a function of daily mean temperature was done with SigmaPlot 9.0 (Systat Software, Richmond, CA, USA).
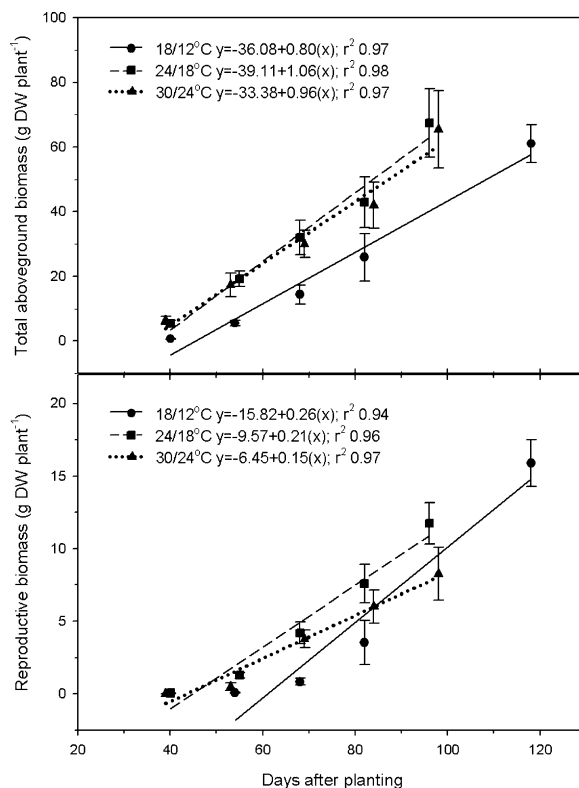

Fig. 1. Response of total aboveground and reproductive biomass accumulation of cuphea to temperature as a function of DAP. Values are means ± S.E., $n = 6$ plants.

3. Results and discussion

The growth of cuphea plants under all temperature regimes was relatively slow between 0 and 40 DAP. Growth of plants dramatically increased and proceeded linearly upon entering reproductive phase (Fig. 1). For the 18/12, 24/18, and 30/24 °C treatments, the first open flowers were observed at 45, 40, and 42 DAP, respectively. The growth rate of plants in each treatment was estimated from the slopes of the linear regression of growth versus DAP as shown in Fig. 1. With respect to the treatments, total biomass growth rate was greatest at 21 °C mean temperature (24/18 °C treatment) and in comparison, was only 10% less at 27 °C (30/24 °C) and was 25% less at the lowest temperature. The effect of temperature on total biomass growth rate was not significant (F value = 1.37), indicating that overall growth of cuphea is relatively adaptable over a wide temperature range.

Temperature did significantly impact reproductive biomass growth rate (F value = 6.16; $P = 0.003$), which decreased linearly with increasing mean daily growth temperature (Fig. 2). Compared to the lowest tempera-
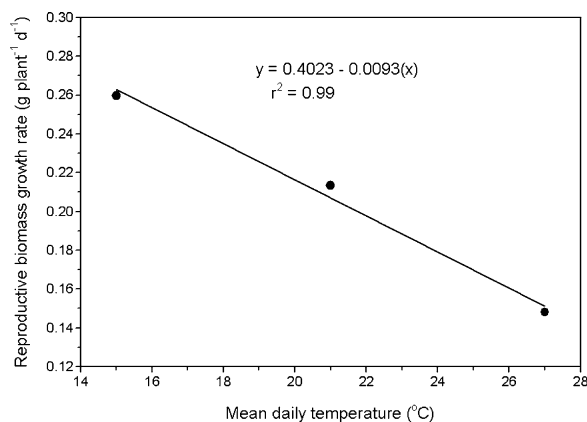
Fig. 2. Growth rates for reproductive biomass accumulation as a function of mean daily growth temperature. Values are slopes based on linear regressions shown in Fig. 1. Growth rates followed a linear decline with temperature.

ture treatment, the growth rate of reproductive tissues was 18% less under the 24/18 °C temperature treatment and was 43% lower under the 30/24 °C treatment (Fig. 2). This high temperature sensitivity may be critical to seed production. Forcella et al. (2005) found that cuphea seed yields decreased with increasing growing season (April through September) temperatures even though aboveground biomass changed little.

In this study, reduced growth rates of reproductive biomass were mainly due to lower flower numbers, as seed production was small due to low self-fertility. Because of this, it should be cautioned that results from this study may not accurately represent field performance where presumably pollinators (e.g., winged insects) would be present. Furthermore, lack of fertility may have affected vegetative biomass growth, although presumably this may occur in the field as well. Nevertheless, results indicate that reproductive development of cuphea is adversely affected by high temperatures.

Leaf photosynthesis acclimated relatively well over the temperature range studied. The highest photosynthetic rates were observed in plants grown under the 24/18 °C treatment (Fig. 3A). The optimum daily growth temperature for photosynthesis predicted by a quadratic function fitted to the data was approximately 23 °C (Fig. 3A). Water use efficiency of CO_2 assimilation decreased by 43% with an increase in maximum growth temperature to 24 °C and remained about the same at 30 °C, which was due mainly to increased transpiration (Fig. 3B). Plant water availability also may be a critical factor for successful cuphea production. Water use efficiency of seed production in field-grown cuphea also tends to be low (Sharratt and Gesch, 2004).
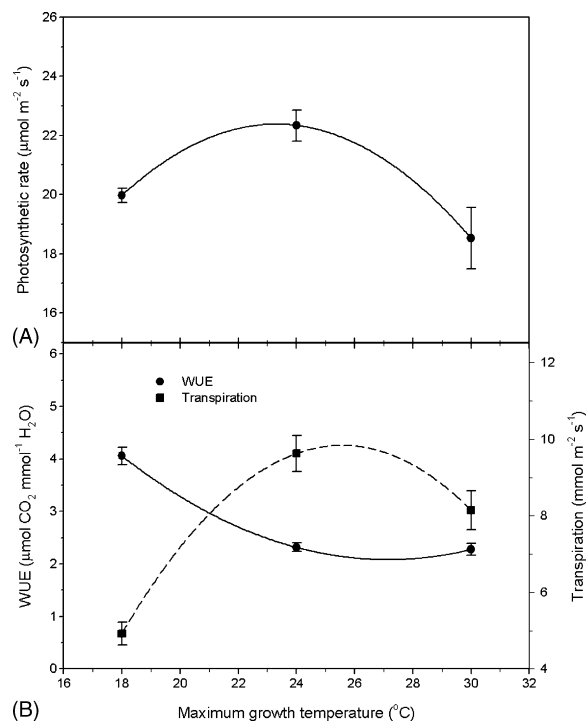

Fig. 3. Leaf photosynthesis rate (A) and water use efficiency of CO_2 assimilation (WUE) and transpiration rate (B) at 40 DAP measured at maximum growth temperature. Values are means ± S.E., $n = 6$. Data were fitted to quadratic functions.

The adaptability of cuphea's vegetative growth is in part attributed to its ability to adjust its photosynthetic capacity with changing temperature. As assessed by the response of photosynthesis to internal CO_2 concentration (C_i) (Fig. 4), both Rubisco-limited (initial slope) and RuBP-limited rates (maximum rate) of photosynthesis increased with decreasing temperature. Compared to the highest temperature treatment, maximum Rubisco activity (V_{cmax}) and maximum electron transport rate of RuBP regeneration (J_{max}) were 76 and 111% greater, respectively, for leaves at 18/24 °C (Table 1). This response is common among plant species that acclimate well to low temperatures (Berry and Björkman, 1980) and helps

Table 1
Effect of growth temperature on apparent *in vivo* maximum Rubisco activity (V_{cmax}) and maximum electron transport rate for regeneration of RuBP (J_{max}) at 48 DAP

48 DAP	Day/night temperature treatment		
	18/12 °C	24/18 °C	30/24 °C
V_{cmax} (μmol m^{-2} s^{-1})	79 ± 2.8	56 ± 3.4	45 ± 2.2
J_{max} (μmol m^{-2} s^{-1})	255 ± 7.2	171 ± 11.8	121 ± 6.0

Values are means ± S.E., $n = 3–4$.
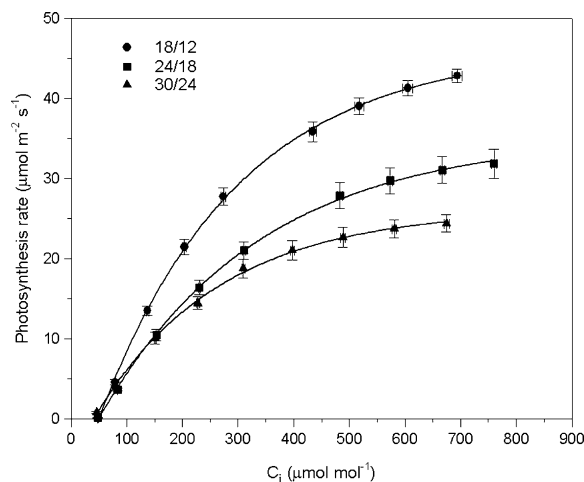
Fig. 4. Leaf photosynthesis as a function of internal CO_2 concentration for the temperature treatments measured at 48 DAP. Values are means of photosynthesis $\pm$ S.E. as a function of mean $C_i \pm$ S.E., $n = 3$–4, and were fitted to an exponential function of the form: $y = y_0 + a(1 - e^{-bx})$, where y equals photosynthesis and x equals C_i.

compensate for constraints placed on the kinetics of key photosynthetic enzymes by low temperatures (Leegood and Edwards, 1996).

In summary, photosynthesis and hence, vegetative biomass production of cuphea (PSR23) appear to adapt to a wide temperature range. However, seed production presently may be better suited to climates with cool to moderate growing season temperatures because of reduced reproductive development under high temperatures. Further research is needed to elucidate the physiological basis for cuphea's heightened sensitivity of reproductive development to high temperatures.

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