CONE THERMOGENESIS AND ITS LIMITS IN THE TROPICAL CYCAS MICRONESICA (CYCADACEAE): ASSOCIATION WITH CONE GROWTH, DEHISCENCE, AND POST-DEHISCENCE PHASES

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Key words: Cycas micronesica; Cycadaceae; cycads; heat stress; inverse calorimetry; simulations; pollination; temperature; thermogenesis.

Many investigators have documented the occurrence of thermogenesis in the reproductive organs of several species within basal angiosperm families as well as in palms, aroids, and lotuses, and in cones of many cycad species (Cycadales) and of some pines (Tang, 1987, 1993; Azuma et al., 1999; Dieringer et al., 1999; Thien et al., 2000; Seymour et al., 2003; Takács et al., 2009). Some plants increase their aerobic metabolism and activate the cyanide resistant pathway or uncoupling proteins (Ito, 2009). Some plants increase their aerobic metabolism and activate the cyanide resistant pathway or uncoupling proteins (Ito, 2009). Some plants increase their aerobic metabolism and activate the cyanide resistant pathway or uncoupling proteins (Ito, 2009). Some plants increase their aerobic metabolism and activate the cyanide resistant pathway or uncoupling proteins (Ito, 2009). Some plants increase their aerobic metabolism and activate the cyanide resistant pathway or uncoupling proteins (Ito, 2009). Some plants increase their aerobic metabolism and activate the cyanide resistant pathway or uncoupling proteins (Ito, 2009).

Conclusions: Cycas micronesica pollen cones exhibit several thermogenic attributes not reported in other cycads, including continuous thermogenesis for many weeks. These cones grow in a hot tropical environment that likely confines their metabolism-generated temperature increases to a small thermogenic window beyond which they encounter heat stress. These findings suggest the presence of thermogenic functions not strictly related to pollination and a potential vulnerability to warming climates.

Key results: Pollen cones exhibit a continuous, but small, metabolically generated thermogenesis for multiple weeks, including a single thermogenic peak temperature greater than peak ambient each day. The magnitudes of those daily peak temperature elevations above ambient reach maxima twice during cone development: a few days before dehiscence and approximately 1 wk post-dehiscence. Excised cones in dark, fixed temperature environments generated multiple thermogenic events (~24 h period) over ~10 d. Cones appear to initiate a protective temperature regulatory response at temperatures ≥38°C.

Methods: We assayed thermogenesis in Cycas micronesica, Guam’s endangered cycad, over successive cone developmental phases by measuring temperatures in shaded and unshaded in situ cones for up to 7 wk. We also studied the effect of ambient conditions on cone thermogenesis in laboratory experiments and estimated the cones’ metabolic heating rates.

Premise of the study: Thermogenesis is a prominent pollination-related feature of cycad cones and is generally assumed to play a role in pollination. Although typically studied just before, during, and immediately after the cones’ pollination phase, thermogenesis may be present in other cone developmental phases.

Plant reproductive organs progress through their various developmental phases. Previous research efforts have led to significant advances in understanding thermogenesis, particularly in angiosperms; e.g., Symlocarpus foetidus Salisb. (Araceae; skunk cabbage) (Knudson, 1974), Philodendron selloum K. Koch (Araceae) (Nagy et al., 1972), Nelumbo nucifera Gaertn. (Nelumboaceae; sacred lotus) (Seymour and Schultz-Motel, 1996, 1997), and Dracunculus vulgaris Schott (Araceae; dragon lily) (Seymour and Schultz-Motel, 1999). Investigators have identified specific functions of thermogenesis in some angiosperms, including protection from freezing (skunk cabbage; Seymour and Blaylock, 1999) and the provision of energy to pollinating scarab beetles, Cyclocephala colasii Endrödi, in the arum lily, Philodendron solimoensis A.C. Sm. (Seymour et al., 2009).

Studies of thermogenesis in several cycad species, mostly within the family Zamiaceae (Tang, 1987; Donaldson, 1997; Seymour et al., 2004; Terry et al., 2004a, b; Roemer et al., 2005, 2008, 2012a, b; Suinyuy et al., 2012; Wallenius et al., 2012), have provided insights into their pollination-related processes. Most such studies have concentrated on the days during and immediately preceding the cones’ dehiscence or receptive phase, a period within which the cone’s maximum temperature elevations have generally been recorded. Given the endangered status of nearly half of the ~300 worldwide cycad species (Donaldson, 2003), furthering our understanding of the cones’ developmental and pollination processes is important for forming appropriate conservation management strategies. Toward this end, studying the thermal behavior of cones over longer
time periods than those immediately adjoining the pollination phase may reveal information important for elucidating the specific roles of thermogenesis at various phases of the cones’ development. Although several Zamiaceae species have been investigated, detailed studies of thermogenesis in specific Cycadales species have been limited, especially for in situ, tropical Cycas (Cycadaeae) species (e.g., Tang, 1987; 1993; Roemer et al., 2012b). Thermogenic plants in hot tropical climates have a small thermogenic window within which they can increase their metabolic heating before they are likely to reach temperatures known to disrupt normal metabolic processes and/or damage plants (Sharkey and Schrader, 2006). This heat stress constraint could make thermogenic tropical species behave quite differently than thermogenic species inhabiting cooler, more temperate-like climates, and this constraint could become even more restrictive when global warming reduces the size of tropical cones’ already small thermogenic windows.

One such tropical species is Cycas micronesica K.D. Hill (Cycadaeae) of Guam, the only native gymnosperm in the Mariana Islands. This species has changed in status from a healthy, vibrant dominant forest species to endangered in less than 6 years due to invasive pests (Aulacaspis yasumatsui first noted in 2003 [Donaldson, 1995; Marler and Muniappan, 2006] and Chilades pandava in 2005 [Moore et al., 2005]). These combined threats have resulted in 92% mortality during the period beginning just before the A. yasumatsui invasion and 2011 (Marler and Lawrence, 2012). A recent study has shown that C. micronesica cones progress through several distinct developmental phases (Marler and Dongol, 2011). Pollen cones proceed through: an initial linear growth phase at the end of which they attain ~50% of their final length and almost all of their girth, an extended, slow/no growth phase lasting ~30 d, a rapid elongation phase in which the cones can double in length over several days (while their girth remains unchanged) and the cones’ rows of sporophylls separate, a pollen dehiscence phase lasting ~3 days, and a final, post-dehiscence period before dehiscence. While those developmental phases have now been well characterized, little has been reported on thermogenesis of C. micronesica beyond the preliminary observations that cones are thermogenic, some for several weeks (Roemer et al., 2012b).

To better understand the potential functions and dynamics of thermogenesis in C. micronesica, we have covered in situ cones with shading to eliminate direct solar heating effects and continuously recorded the cones’ temperatures for multiple weeks. This approach has allowed us to isolate and investigate the timing, duration, and magnitudes of cone thermogenic events present during the cones’ developmental phase progression, starting during the slow/no growth phase and proceeding through post-dehiscence. Because cones grow from the apex of the stem of tall trunked trees, and some trees grow up to ~6 m high in habitats where C. micronesica is the emergent canopy species (Fig. 1), cones can be exposed to significant solar heating. Thus we also examined the temperature responses of cones that were directly exposed to solar radiation in this humid, tropical island climate. We further explored pollen cones’ thermogenic behavior under controlled laboratory conditions using excised cones. Characterizing C. micronesica cones’ thermogenic patterns may help to structure future studies of the role of thermogenesis in this species and improve our understanding of the relationship of thermogenesis to the release of cone volatiles, including how volatiles and thermogenesis influence the putative pollinator, a moth in the genus Anatrachynts (Lepidoptera: Cosmopterigidae) (Terry et al., 2009; Marler, 2010). In addition, studying cones of a tropical cycad will be useful in obtaining data for comparison with the thermal responses of cones of cycads that are native to cooler climates. Finally, studying members of the basal Cycas genus should provide increased understanding of various facets of contemporary plant biology (Brenner et al., 2003a, b).

MATERIALS AND METHODS

Coney—Cycas micronesica is endemic to the islands of the western Pacific Ocean, and it thrives on all common soils throughout these islands. Our study was restricted to a population growing at Ritidian Point, Guam, on the coralline Ritudin soil series that are Clayey-skeletal, gibbistic, nonacidic, isohyperthermic Lithic Ustorthents (Young, 1988). In the June and July coning seasons of 2007, 2008, and 2012, we measured temperatures, dimensions, and pollen dehiscence percentages of in situ cones, and performed laboratory experiments in which cone temperatures were measured. For the multiple-week long in situ field measurements, we selected pollen cones that had passed their early phase of rapid linear growth and were already within their ~30-d slow/no growth phase (Marler and Dongol, 2011). Pollen cones retain an ovoid shape (Fig. 2) during their successive slow/no growth, rapid elongation, dehiscence, and post-dehiscence phases. Pollen cones were studied in the field on intact plants and in the laboratory with excised cones. The 32 pollen cones studied had a mean diameter (±SD) of 9.6 ± 1.5 cm and length of 24 ± 5.6 cm at the time of each cone’s recruitment.

We also measured temperatures on a small number of ovulate reproductive structures (the aggregations of megasporophylls bearing ovules, herein referred to as ovulate cones) that were only studied for short periods in situ to avoid interfering with seed set. Ovulate cones change their shape dramatically as they progress from initially being a sphere of tightly overlapping sporophylls, that gradually loosen, spread out and become completely separated and open to the ambient air (Marler and Dongol, 2011). The six ovulate cones studied had initial mean diameter of 10.4 ± 1.3 cm and length of 11.5 ± 3.3 cm.

Temperature and light measurements—To measure temperatures, we inserted a calibrated thermistor probe (Onset Computer Corp., Pocasset, Massachusetts, USA; TCM50-HA) ~3 cm deep into a small hole bored between sporophylls at approximately mid-height of each cone. Each probe was held in place by several loops of electrical tape that were wrapped around the cone and overlapped the probe where its leads exited the cone. Those leads were covered with insulating foam and aluminum foil to reflect solar radiation. Those methods ensured that probes were always properly held in place, as verified by regular inspection (daily in almost all cones). Onset HOBO H8 Outdoor/Industrial External Data Loggers recorded the thermistor temperatures every 2 min. The use of one probe per cone in a standardized configuration was possible for pollen cones due to their fixed ovoid shape during all developmental phases studied. In contrast, the ovulate cones’ markedly changing geometries precluded measuring a single “typical” cone temperature at one location throughout their developmental phases. Thus, ovulate cones’ temperatures were measured only during the cones’ early, spherical configuration period. Cone temperatures were averaged over 10-min periods to minimize noise effects, while ambient temperatures were averaged over 60-min periods to smooth out short-term fluctuations in ambient temperature. For one laboratory experiment, we placed a type K thermocouple in a pollen cone and recorded its temperatures with a portable data acquisition recorder (Fluke, Everett, Washington, USA, Model 54II). We measured photon flux density (PFD) values with a Skye SKP 200/215 PAR quantum sensor (Skye Instruments, Llandrindod Wells, Powys, UK).

Field measurements—We measured temperatures in 13 pollen cones and four ovulate cones that were shaded from solar radiation by large, thick tarpaulins or beach umbrellas. This shading isolated the effects of the cones’ metabolic contribution to their temperature elevations, but still allowed ventilation and pollinator access. We also measured the temperatures of 10 unshaded in situ pollen cones and two unshaded in situ ovulate cones that were located in open areas that exposed the cones to direct solar radiation. Those unshaded cones were further exposed to the sun by removing all branches and leaves immediately above and around the cones. The ambient temperature was measured close to each cone, with the ambient probe always well shaded and covered with aluminum foil to reflect any stray solar radiation. When a field cone of appropriate developmental phase was found during field surveys, a thermistor
probe was placed in the cone, and measurements were initiated. The timing of a cone’s development within its ~30-d slow/no growth phase could not be determined exactly for each new cone. Thus some cones studied had very lengthy, close to 30-d periods of data recorded during their slow/no growth phase, while others had only a few such days; likewise, during the post-dehiscence phase, some cones were measured for up to 2 wk, but for only a few days on others. Consequently, although all 13 cones provided temperature data for all of the days during and closely adjoining elongation and dehiscence, the total number of days studied before elongation and after dehiscence varied from cone to cone.

In seven of those 13 shaded field cones, we also measured each cone’s length, circumference, and percentage of pollen dehiscence daily as the cones proceeded from elongation through post-dehiscence. Once one of those seven cones began loosening at its base, an event that presaged the imminent start of the rapid growth phase, six sporophylls were excised daily at ~08:30–09:30 hours (two each from the cone’s top, middle and bottom, with care taken not to remove sporophylls near the temperature probe), and each sporophyll was examined to determine the percentage of sporangia that were open and dehiscing pollen. Sporophyll removal continued until the day when 100% dehiscence was reached on all six sporophylls. For three of the six remaining shaded cones, we monitored both their dimensions and their developmental status (separation of sporophyll rows/presence of dehisced pollen) daily or every other day, but exact dehiscence values were not counted. Finally, the remaining three shaded cones had their developmental status similarly monitored, but no length or diameter measurements were made.

The die effect of metabolic heating was quantified by determining each shaded cone’s daily peak temperature elevation, i.e., the difference between the cone’s peak temperature and that day’s maximum ambient temperature (as measured near that cone). Since the developmental phases of cones were asynchronous,
and since data were taken over multiple years, it was necessary to develop a method of aligning the daily results from all cones. To do so, for each cone, “day 1” was assigned as the day on which that cone’s dehiscence percentage (averaged over all six sporophylls) first reached 10% in the seven cones for which sporangia were counted: for the other six cones day 1 was the first day on which pollen was visually observed. The earliest and latest normalized days had results from only three cones, with intermediate days all having data from three or more cones, with the maximum number of cones being 13.

**Laboratory experiments**—Pollen cones were excised during their slow/no growth phase, and their thermogenic activity was examined under controlled ambient conditions. Excised pollen cones have been used for laboratory studies since they are known to generate multiple thermogenic events similar to those seen in the field (Tang, 1987; Roemer et al., 2005, 2008, 2012a; Terry et al., 2007). Although excised pollen cones do not go through the full elongation seen in situ, their rows of sporophylls do loosen, and their sporangia dehisce pollen (personal observations). We performed three types of laboratory experiments to determine the influence of different ambient temperature and light conditions on pollen cones’ temperatures.

First, to determine whether cones would generate their daily temperature increases in the absence of any changes in ambient light or temperature, we placed cones in complete darkness and at a fixed ambient temperature. Two cones were tested inside an incubator regulated at 32.6 ± 0.25°C (approximating the average daily ambient in situ high temperature) during 10 d, and two cones were tested at 24.7 ± 0.25°C (approximating the average daily ambient in situ low temperature) during 24 d. The lower temperature tests were performed in a temperature-controlled laboratory, with the cones placed inside a covered, insulated, but ventilated, box that blocked out room light and minimized ambient temperature fluctuations.
Second, to determine whether the timing of the cones’ daily temperature peaks was affected by the timing of the daily ambient temperature cycle, we exposed four cones to an ambient temperature cycle that was significantly delayed past the normal quotient outdoor ambient temperature timing. To obtain this delayed cycle, we placed cones in a darkened (midday light level of ~10 ± 4.2 μmol-m⁻²-s⁻¹) interior room of a small building with no temperature control. The room’s ambient temperature cycle thus passively followed, but was delayed behind and lowered in magnitude from, the outside ambient temperature cycle. The Cycas micronesica habitat typically reached its highest daily temperature of ~33°C at 13:10 ± 00:45 hours as measured for 96 test days. The interior room, in contrast, reached its highest daytime interior ambient temperature (28.1 ± 0.7°C) much later, at 17:45 ± 01:05 hours. The room’s nighttime low temperature was 25.7 ± 0.8°C.

Finally, to test a cone’s response to a high ambient temperature under controlled laboratory conditions, we exposed one excised cone to three successive days of alternating hot/cold, day/night ambient temperatures of 26°/15°C, 40°/15°C, and 26°/15°C by placing it in a refrigerator at ~15°C for ~12 h overnight and into either a laboratory room at 26°C, or a temperature controlled incubator at 40°C during the day. That cone was excised just as it began its rapid growth phase, thus ensuring that it would undergo several days of substantial thermogenic events.

Estimation of cone metabolism—We used the inverse calorimetry method (Roemer et al., 2012a) to estimate the magnitudes of the cones’ metabolic heating rates. That approach, an expanded version of one first used by Knutson (1974) on thermogenic skunk cabbage inflorescences, takes advantage of the measured cone and ambient temperatures by using them to estimate the cones’ metabolic heating rate, \( QMc \), where \( \dot{Q} \) is the cone’s thermogenic metabolism (W), \( M \) is the cone’s mass (kg), and \( c \) its specific heat (J·kg⁻¹·°C⁻¹). In that method, the cones’ metabolic heating rate calculation is based on conservation of energy for the cone, which gives

\[
\frac{\dot{Q}^\text{con}(t)}{M_c} = \frac{dT^\text{con}(t)}{dt} - \frac{(T^\text{con}(t) - T^\text{core}(t))}{\tau}.
\]

(1)

Here, \( T^\text{con}(t) \) and \( T^\text{core}(t) \) are the measured ambient air and cone temperatures (°C), \( t(s) \) is time, and \( t(s) \) is the cone’s thermal time constant. In the present study, in which only rough estimates of the metabolic heating rates are presented, a time constant of 120 min (as estimated from the field and laboratory cones) was used for all cones since they were all close to the same diameter, and both the laboratory cones and the in situ shaded cones were in similar low velocity ambient air environments (Hamada, 2012). That value of the time constant was then used in Eq. 1 to calculate the metabolic heating rate when also using the cone and ambient temperatures as measured every 2 min for all days of all of the shaded pollen cones. That calculation always involved both the temporal derivative of the cone temperature and the noisy ambient temperature measurements, both of which produce noise in the estimates of the metabolic heating rate. Thus, the sequential 2-min estimates of \( QMc \) were averaged over 60-min periods, giving results that retained the major trends in the metabolic heating rate but smoothed the effects of noise.

RESULTS

Shaded field cones—The shaded pollen cones’ had peak temperature elevations due to metabolic heating every day over a multiple week period, encompassing every developmental phase studied, and the magnitudes of those elevations changed as a function of developmental phase (Fig. 3). Those daily peak temperature elevations began as early as 25 d before pollen dehiscence and persisted until a little more than a week past the end of dehiscence, with (average) elevations reaching small maxima of ~4°C on two widely separated days, one just before dehiscence and one approximately a week post-dehiscence. In addition, the shape of the cones’ daily temperature vs. time curve changed with developmental phase (Fig. 4): progressing from initially (during the slow/no growth period) being thermogenically elevated above ambient most of each day—except during an ~2–3 h sunrise period of rapid ambient temperature increase when cones were heated by both the air temperature and by cone metabolism (Fig. 4A, B); to then remaining above ambient for all 24 h of each highly thermogenic day (Fig. 4C) during the rapid growth and dehiscence phases; to finally just tracking the ambient temperature during the late post-dehiscence phase when cone metabolism had essentially ceased (Fig. 4D).

The estimated metabolic heating rate \( QMc \) values of the 13 shaded pollen cones changed as the cones progressed through their developmental phases (Fig. 5). The cones’ diel-peak 24-h average \( QMc \) values illustrate how thermogenic heating is present over many weeks of the cones’ developmental phases, with the metabolic heating rate varying with cone developmental phase, including bimodal maxima as were seen in the cones’ peak temperature elevation results.

The shaded pollen cones’ diel thermogenic temperature peaks always occurred later than their ambient peak temperatures (Fig. 6; also see Fig. 4). That time difference was minimal at the beginning of the slow/no growth phase, slowly increased to a maximum near the day when the maximum post-dehiscence temperature difference occurred, and then rapidly decreased during the subsequent days. Specifically, on the day of their maximum post-dehiscence temperature elevation, the maximum cone temperature occurred at 16:35 ± 00:50 hours, 4 h later than the peak in ambient temperature at 12:25 ± 01:15 hours.

The shaded ovulate cones typically exhibited the same diel temperature vs. time shape as was present in the males’ slow/no growth phase of Fig. 4A, with metabolic heating providing an ~1°C–2°C cone temperature elevation contribution for the days before the overlapping sporophylls began to loosen and unfold.

Unshaded field cones—The unshaded field cones reached higher peak temperature elevations than the shaded cones since they were simultaneously heated by both cone thermogenesis and solar irradiation. Of 120 cone-days on which the unshaded pollen cones had peak cone temperature elevations ≥1°C above peak ambient, 45 cone-days reached maximum temperatures
for several hours temporarily increased the cone’s temperature to a maximum each day on at least 11 successive days. The other three cones exposed to constant ambient temperatures also showed such baseline trends and diel peaks. The two cones tested at 24.7°C had a combined total of 24 sequential peaks with an average peak temperature elevation of 3.2°C ± 0.8°C ≥38°C, with those high temperatures detected in 8 of the 10 unshaded cones. In contrast, for the shaded pollen cones, of 181 cone-days with peak cone temperature elevations ≥1°C above peak ambient, only one cone ever reached a maximum temperature of ≥38°C and that only occurred on one day with $T_{\text{max}} = 38.6°C$. Most interestingly, on 15 of the 45 cone-days when the exposed cones reached ≥38°C, five cones exhibited what appeared to be protective thermoregulatory behavior by presenting a peak cone temperature vs. time curve that was flat for many hours as shown in Fig. 7 for an example cone. On all days before and after its flat responses, that cone had temperature maxima <38°C and temperature vs. time curves that displayed the typical, single rounded temperature peak shape (e.g., Fig. 4). The flat temperature response behavior was never seen on any of the 75 cone-days on which the exposed pollen cones had $T_{\text{max}} <38°C$, nor on any of the 181 shaded cone-days, all of which had temperature vs. time curves with the rounded, single temperature peak shape.

**Excised cone studies**—The excised pollen cone exposed to daytime ambient laboratory temperatures of 26°C, 40°C, and 26°C on successive days had lengthy, steady peak temperature elevations above ambient of 3.6°C, 1.8°C, and 3.6°C, respectively, with the cone remaining between 41.5°C and 42°C for nine consecutive hours on the 40°C day. This behavior again suggested high temperature protective regulation by the cone. Excised pollen cones maintained at 24.7°C in darkness revealed a baseline metabolism that elevated the cones’ temperatures above ambient temperature at all times (Fig. 8). In addition to this baseline metabolism, a metabolic enhancement lasting for several hours temporarily increased the cone’s temperature to a maximum each day on at least 11 successive days. The other three cones exposed to constant ambient temperatures also showed such baseline trends and diel peaks. The two cones tested at 24.7°C had a combined total of 24 sequential peaks with an average peak temperature elevation of 3.2°C ± 0.8°C ≥38°C, with those high temperatures detected in 8 of the 10 unshaded cones. In contrast, for the shaded pollen cones, of 181 cone-days with peak cone temperature elevations ≥1°C above peak ambient, only one cone ever reached a maximum temperature of ≥38°C and that only occurred on one day with $T_{\text{max}} = 38.6°C$. Most interestingly, on 15 of the 45 cone-days when the exposed cones reached ≥38°C, five cones exhibited what appeared to be protective thermoregulatory behavior by presenting a peak cone temperature vs. time curve that was flat for many hours as shown in Fig. 7 for an example cone. On all days before and after its flat responses, that cone had temperature maxima <38°C and temperature vs. time curves that displayed the typical, single rounded temperature peak shape (e.g., Fig. 4). The flat temperature response behavior was never seen on any of the 75 cone-days on which the exposed pollen cones had $T_{\text{max}} <38°C$, nor on any of the 181 shaded cone-days, all of which had temperature vs. time curves with the rounded, single temperature peak shape.

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The four laboratory pollen cones exposed to the delayed peak ambient temperature exhibited 62 thermogenic events with an average peak temperature elevation above the peak ambient temperature of 2.2 °C ± 1.0 °C (max. 4.7 °C). The ambient laboratory temperature peaked at 17:45 ± 01:05 hours, which was significantly later than the typical in situ ambient temperature’s peak near 13:10 ± 00:45 hours. Similarly, those four laboratory cones’ peak temperatures occurred at 19:15 ± 00:45 hours, again a much later time than the in situ shaded cones’ peak time of 14:30 ± 01:50 hours, as averaged over all cone development phases.

**DISCUSSION**

These field measurements and laboratory experiments on *Cycas micronesica* cones have uncovered evidence for several thermogenic features of pollen cones not reported in other cycad species including an extended, multiple week period of continuous thermogenic activity; a day-to-day peak temperature elevation pattern with bimodal maxima, one maximum a few days before dehiscence and one ~1 wk post-dehiscence; and possible protective thermoregulation at high cone temperatures (exceeding ~38 °C). In addition, the present results showing the entrainment of the pollen cones’ temperature peaks by the delayed ambient temperatures are consistent with those from previous research on *M. lucida* and *M. macleayi* pollen cones wherein the timing of cones’ diel temperature peaks was found to be strongly affected by the timing of the ambient temperature.

![Figure 6](image1.png)

**Fig. 6.** Difference (minutes) between the time of the diel temperature peak and the time of the diel ambient temperature peak vs normalized day for cones of *Cycas micronesica*. Results are averaged over all 13 shaded in situ pollen cones, and days are normalized as in Fig. 3. All days shown have average values from at least three cones, with days near day 1 showing the averaged data from all 13 cones.

![Figure 7](image2.png)

**Fig. 7.** Temperature vs. time of day for an exposed in situ pollen cone of *Cycas micronesica* that showed putative protective temperature regulation against high temperatures for several hours on each of three successive days (A, B, C) with cone temperatures ≥ 38 °C.
cycle (Roemer et al., 2008). The present results are also consistent with prior observations that cycad cones generate multiple temperature peaks with intervals close to, or less than, 24 h when the ambient temperature is constant and cones are kept in a fixed dark or light condition (Roemer et al., 2008), or when they are exposed to changing dark-to-light conditions at a fixed ambient temperature (Tang, 1987, 1993).

**Lengthy predehiscence thermogenesis**—The persistence of thermogenesis over several cone developmental phases observed in these *C. micronesica* pollen cones has not been noted in other cycad species. Prior cyclad research has only examined cones for evidence of thermogenic events for ~2 wk or less around the dehiscence period (Tang, 1987; Donaldson, 1997; Seymour et al., 2004; Terry et al., 2004a, b; Roemer et al., 2008, 2012a; Suinyuy et al., 2012), except for anecdotal evidence for some early, predehiscence thermogenesis in *Macrozamia macleayi* (Terry et al., 2004b). Thus, whether cones of other species have a prolonged thermogenic period during predehiscence, or over several developmental phases, is not known. In angiosperms the longest thermogenic period reported has been for ~2 wk in skunk cabbage (Knutson, 1974), wherein continuous (24 h) thermogenic heating apparently prevents floral freezing (Seymour and Blaylock, 1999).

For *C. micronesica*, the function of the enhanced thermogenic metabolism during the cones’ long, slow/no growth phase is unclear since no significant visible changes in the cones are evident even though this phase lasts for ~30 d. Nor are insects attracted to the cones during this time, at least until the cones begin to loosen at the beginning of the rapid growth phase. Clearly, a metabolically intensive process (a typical metabolic heating rate of 0.015°C/min (Fig. 5) corresponds roughly to 1 W for a 1 kg cone) is occurring, possibly in preparation for the cones’ upcoming developmental phases. Several functions can be postulated for this increased metabolic activity, including cell division without expansion or elongation and/or biochemical transformations priming the cells for one or several future activities such as production of volatiles/volatile precursors for attracting pollinators, rapid cone elongation, formation of sporangia and pollen, and/or the formation and storage of starches for aiding in the increased thermogenic activity that occurs at the end of the no growth period.

**Bimodal peak temperature elevation maxima**—The first maximum occurs near the beginning of the cones’ rapid elongation phase, and before significant pollen dehiscence begins (Fig. 3). Thus, this first maximum has a closer temporal association with cone elongation than with the onset of dehiscence. Other pollination-related activities are also present at this time; in particular, the cones begin to emit volatiles and attract insects just before dehiscence (L. I. Terry et al., unpublished manuscript).

The second peak in cone thermogenic activity occurs almost 1 wk after complete dehiscence, but with some pollen grains still remaining on adaxial sporophyll surfaces directly beneath the sporangia. At this time, cones still emit volatiles and attract *Anatrachynis* moths (L. I. Terry et al., unpublished manuscript) to the pollen cones where the adults breed and larvae feed and develop. This second maximum could thus be part of a week-long, post-dehiscence period in which pollination-related activities are still ongoing. These elevated temperatures may also benefit adult moths and larval development.

Finally, if the two maxima in thermogenic activity do indeed have two independent functions, then the intervening decrease in the cones’ peak temperature elevation during dehiscence could be a result of an overlap interval wherein one activity is present but diminishing, and the other is present but just starting, rather than a temporary diminishment of a single metabolic process. The decrease in the cones’ peak temperature elevation during dehiscence is not likely an artifact caused by the separation of sporophylls during cone elongation since there was also a subsequent, even larger, increase in the post-dehiscence peak temperature elevation that occurred while the sporophyll rows were still separated. In addition, the rows of sporophylls just above and below the probe were always covered by tape as was their inter-row separation, thereby stabilizing convective heat losses during cone lengthening. A peak temperature elevation pattern involving bimodal maxima, as observed in *C. micronesica*’s cones, has not been reported in other cycad species, but this may just be the result of a lack of studying cones over longer periods as stated already. In comparison to *C. micronesica* cones, during their pollination phase the cones of *M. macleayi* and *M. lucida* have large daily cone peak temperature elevations over many days, with a maximum near the day of their peak dehiscence rate (Roemer et al., 2008; R. B. Roemer, unpublished manuscript). Those *Macrozamia* cones’ large daily thermogenic temperature elevations (which can reach up to 15°C above ambient) are also closely associated with the cone’s daily increase in volatile emissions and the en masse movement of their obligate thrips pollinator out of the cone. Such an immediate temporal relationship of thermogenesis with pollinator activity is not present in the *C. micronesica* cones.

**High temperature protective regulation**—Temperature regulation has not been reported in cycads except for preliminary speculations (Roemer et al., 2012b). The physiological mechanism underlying the putative regulation seen in the present study of in situ unshaded cones that reach >38°C and of a laboratory cone exposed to ~40°C could be some combination of decreased thermogenic metabolism and increased evaporation. Metabolic decreases have recently been measured in laboratory respirometry experiments with *M. macleayi* cones in which there was a sudden decrease in oxygen uptake when the cones’ temperatures approached ~40°C (R. B. Roemer, et al.,

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**Fig. 8.** Cone temperature vs. experimental day for an excised pollen cone of *Cycas micronesica* in the laboratory in the dark at a fixed ambient temperature of 24.7°C ± 0.25°C. Day 0 is the day the cone was excised and placed into the dark, fixed ambient temperature environment. Some of the ambient temperature fluctuations present are caused by the air around the cone being heated by the cone’s increased metabolism.
unpublished manuscript). Similar metabolic changes could be active in *C. micronesica* cones. For example, in the cone exposed to the 26°C/40°C/26°C daytime laboratory temperatures, a halving of the cone’s metabolism during the 40°C ambient exposure compared with that at 26°C ambient would result in the observed halving of the cone’s temperature elevation from 3.6°C to 1.8°C. Such a decrease in metabolism at a high cone temperature would be opposite in direction to that expected from any Arrhenius temperature dependence, in which metabolism would be expected to increase with temperature. Such decreases in metabolism as temperature increases have also been observed in thermogenic angiosperms (Seymour et al., 2010) that appear to be regulating their temperature.

*Cycas micronesica* plants’ high temperature protective responses might be present since they live in a hot tropical environment with an average maximum daily temperature of ~33°C that provides a high baseline temperature upon which thermogenic heating builds. Thus the cones’ thermogenic metabolism magnitudes might be limited to avoid heat stress temperatures (say 40°C). Many plant species limit their temperature elevations by increasing their evaporation rate (Sharkey and Schrader, 2006), and some tropical plants change their geometry to avoid high temperatures (Patil et al., 2002). In most cases, plant tissues are responding to increased ambient, rather than thermogenically generated, high temperatures. For highly active thermogenic plants, the additional mechanism of avoiding heat stress by significantly reducing the plant’s metabolism is also potentially available. Further studies examining the conditions in which heat stress avoidance is implemented in this species may reveal trade-offs between the functional outcomes of the cone’s normal metabolic activity and decreased functionality due to stress related metabolic reductions. Increased evaporation likely also plays a role in avoiding high temperature stress, although in these tropical *C. micronesica* cones its effects would be lessened by the high relative humidity present, particularly during the wet season that coincides with the end of the cone’s main pollination season.

A comparison of the thermogenic characteristics of *C. micronesica* cones to those of *Macrozamia* species cones is of interest since they live in significantly different climates, a factor that likely affects their ability to increase their thermogenic metabolism. In Guam, with its pollination season’s average day/night ambient temperatures of ~33°C/27°C, the *C. micronesica* cones experience high ambient temperatures during the day without significant cooling at night. In contrast, the two *Macrozamia* species cones have their dehisence phase during the spring with day/night ambient temperature averages of ~26°C/14°C in southern Queensland, Australia (Roemer et al., 2005, 2008). Furthermore, many *C. micronesica* cones are exposed to solar radiation with a photon flux density of ~2100 µmol·m⁻²·s⁻¹ at noon during the pollination season. In contrast, in the understory where almost all *Macrozamia lucida* and *M. macleayi* plants reside, the measured noontime photon flux densities during the pollination season are only ~10 ± 4 µmol·m⁻²·s⁻¹. Given the general observation that plants do not function well at temperatures above ~40°C (Sharkey and Schrader, 2006), the *C. micronesica* cones’ tropical climatic conditions may limit the magnitudes of their thermogenically generated temperature elevations. This possibility can be seen in the maximum thermogenically generated peak temperature elevations above peak ambient, and the estimated peak metabolic heating rates for that species’ cones, 6.7°C and ~2.5 W/kg, respectively, compared with *M. lucida* and *M. macleayi* cones whose corresponding values are ~15°C and 25 W/kg (Roemer et al., 2012a). A third *Macrozamia* species, *M. machinii*, studied in Inglewood Forest, Queensland, Australia, at a latitude similar to the Brisbane test sites, has an average maximum ambient temperature in the November coneinge season of ~30°C (range, 26.1°C–34.4°C) that is warmer than the Brisbane test sites and has an average minimum overnight temperature of 15.9°C (range, 14.5°C–17.9°C) that is similar to the Brisbane test sites. These small *M. machinii* pollen cones (with typical masses of ~170 g) exhibit high, diel metabolic heating rates of up to 3.6 W that occur near/after sundown when their beetle pollinator becomes active (Seymour et al., 2004; Terry et al., 2004b). Although they have high metabolic rates and relatively high peak ambient temperatures, the *M. machinii* pollen cones do not seem to reach high thermogenically generated temperatures (e.g., ~38°C), likely since their peak metabolic activity occurs in the evening when the ambient temperature is dropping rapidly and thereby cooling the cones. Last, the relatively high overnight ambient temperatures experienced by the *C. micronesica* cones may contribute to the persistence of their overnight elevated metabolisms through Arrhenius effects—as compared with the three *Macrozamia* species, whose in situ cones’ metabolisms appear to be minimal at night due to extensive cooling by their colder nighttime ambient temperatures.

Finally, the effects of global warming could be very significant for *C. micronesica*, even with moderate ambient temperature rises, thus indicating the need for additional studies on the cones’ putative thermoregulatory behavior. The fact that these tropical cones have a small thermogenic window in which to operate limits the intensity of their thermogenic events. Global warming will shrink that window even more and thus may add yet another stress to these already endangered plants.

**LITERATURE CITED**


