Aquatic Botany xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Respiration, thermogenesis, and thermoregulation of *Victoria cruziana* flowers

Natalie G. Schimpf[a], John S. Terblanche[b], Martin F. Smit[c], Philip G.D. Matthews[a,*]

[a] Department of Zoology, University of British Columbia, Vancouver, BC V6T1Z4, Canada
[b] Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa
[c] Stellenbosch University Botanical Gardens, Stellenbosch, South Africa

ARTICLE INFO

Article history:
Received 24 September 2016
Accepted 30 November 2016
Available online xxx

Keywords:
Victoria cruziana
Flower
Thermogenesis
Thermoregulation
Metabolic rate
Respiration rate
Temperature

ABSTRACT

The flowers of *Victoria cruziana* bloom over two consecutive nights, during which time they dramatically increase their metabolic rate (MR) to raise their internal temperature by 7–10 °C above air temperature. To investigate the metabolic cost of thermogenesis in *V. cruziana* and determine the role of ambient temperature and developmental phase on floral temperature, flowers growing in an outdoor pond were measured *in situ* by placing them within a temperature-controlled, flow-through respirometry chamber. The flower's metabolic rate and temperature were recorded simultaneously over the 40 h of their two-night flowering cycle. During this time, flowers were exposed to either a normal (cool nights, warm days) or an inverted (cool days, warm nights) thermal regime. *V. cruziana* flowers exposed to normal daily temperature variation showed a large increase in MR on the first evening that declined steadily over the subsequent days. Exposure to experimentally-manipulated cooler daytime temperatures did not stimulate flowers to increase their MR, indicating a lack of thermoregulatory capacity. However, rewarming a cooled flower on the second evening caused a large increase in MR, but not in temperature. This increase appeared to arise due to the delayed consumption of energy reserves that would otherwise have been used over the course of the preceding day, coupled with a more open, and less insulated, floral chamber. The pattern of thermogenesis shown by *V. cruziana* is unlike that of its closest relative, *V. amazonica*, which shows large increases in MR and temperature over both nights of flowering.

1. Introduction

The ability of an organism to produce heat is most often associated with warm-blooded animals. But some angiosperms also possess this capacity, dramatically increasing the metabolic rate (MR) of their flowers to elevate their temperature well above that of the surrounding air. While floral thermogenesis is comparatively unusual among the angiosperms, this ability is particularly well developed in some members of the Araceae (aroid lilies), Nymphaeaceae (water lilies), and Nelumbonaceae (sacred lotus) (Seymour and Schultze-Motel, 1997). These flowers generate heat within their tissues by using an alternative oxidase (AOX) pathway in the mitochondria (Elthon and McIntosh, 1987). Thus, heat production results from aerobic metabolism and the MR of the flower, measured indirectly either as oxygen uptake or carbon dioxide release, closely tracks thermogenic activity (Seymour et al., 2015). Although generating sufficient heat to increase the temperature of a flower is energetically costly, from an evolutionary perspective this strategy benefits the flower by providing their insect pollinators with a 'heat reward' in the form of a thermally suitable microclimate (Seymour et al., 2003), enhancing the release and distribution of volatile perfumes to signal to pollinators (Meeuse and Raskin, 1988), or by facilitating early flowering in sub-zero conditions (Knutson, 1974).

The pattern of heat production varies between the flowers of different thermogenic species. Heat production often increases and decreases during particular times of the day or night, coinciding with specific phases of floral development, such as the shedding of pollen (Seymour, 1999). As well as thermogenesis changing with the developmental stage of the flower, some species also possess the ability to up-regulate their heat production in response to decreases in ambient temperature, a central requirement for thermoregulation. A pronounced ability to thermoregulate has been found in the arum lily *Philodendron selloum*, the eastern skunk cabbage *Symplocarpus foetidus*, the voodoo lily *Dracunculus vulgaris*,

* Corresponding author at: University of British Columbia, Department of Zoology, Canada.
E-mail address: pmatthews@zoology.ubc.ca (P.G.D. Matthews).

http://dx.doi.org/10.1016/j.aquabot.2016.11.014
0304-3770/

and the sacred lotus *Nelumbo nucifera* (Seymour et al., 2010a). All of these flowers possess a biochemical feedback mechanism that produces a substantial increase in MR and heat production when the temperature of the flower undergoes a small decrease (Umekawa et al., 2016). Thus, thermoregulatory flowers show an inverse relationship between ambient temperature and MR, enabling them to maintain a relatively stable internal temperature across a range of ambient temperatures. While a strong thermoregulatory response has been found in the sacred lotus and in several aroid species, the temperature regulation abilities of members of the Nymphaeaceae have yet to be established.

The spectacular flowers of the giant *Victoria* water lilies, *V. amazonica* and *V. cruziana,* have long been known to be thermogenic. Both species produce large, night-blooming protogynous flowers that display a two-night flowering sequence (for floral anatomy see Supplementary data Figs. S1 and S2). However, our understanding of the thermogenic and metabolic changes displayed by the flowers of *Victoria* comes almost entirely from studies on *V. amazonica* (Prance and Arias, 1975; Seymour and Matthews, 2006) and is as follows: on the first evening, mature flower buds show a steady increase in temperature and MR in advance of sunset. At sundown the air temperature begins to fall and the sepals and petals open, coinciding with a peak in both CO_2 production rate ($\dot{M}CO_2$, a proxy of MR) and floral chamber temperature. The floral chamber achieves a mean maximum temperature of 34.7 °C when ambient air temperature falls below 25 °C (Seymour and Matthews, 2006). Following this initial thermogenic increase, the MR of the flower decreases throughout the night and following morning. Towards the second evening the flower undergoes another increase in MR, causing the temperature in the floral chamber to again rise above ambient, generally achieving a lower temperature than on the first evening. Despite the highest floral chamber temperatures occurring on the first evening, the maximum MR could occur on either the first or second night of flowering (Seymour and Matthews, 2006). An attempt was made during this 2006 study to also investigate the thermoregulatory capacity of *V. amazonica*, by manually applying ice water over a respirometry hood to cool the flower within. Although floral MR was observed to increase in the single flower exposed to artificially lowered midday temperatures (~23 °C), a lack of access to ice in the field prevented this experiment from being repeated. From this initial work the presence or absence of a thermoregulatory capacity in this species remains an open question.

In contrast to the attention given to *V. amazonica*, only one study has attempted to measure the thermogenic behaviour of *V. cruziana* flowers growing in their native habitat (Valla and Cirino, 1972). Unfortunately, this work was not conducted *in situ*, but on flowers that were collected from wild plants as mature buds and transported to a laboratory where they were measured overnight. This study showed that floral chamber temperature increased to 32–33 °C, or 7.1–7.5 °C above air temperature between 20:00 h and 21:00 h. Two further studies on the thermogenesis and MR of *V. cruziana* flowers have also been undertaken on plants cultivated in glasshouses maintained under more stable air and water temperatures (mean temperatures of 25 °C and 30 °C, respectively) (Lamprecht et al., 2002a,b). Tracking floral temperatures over two days again revealed a peak in floral temperature on the first evening at 19:30 h, but on the following day there also appeared to be a second temperature peak that could occur in either the morning or afternoon (Lamprecht et al., 2002a,b). This pattern is in contrast to the regular nocturnal temperature increases observed in *V. amazonica* on both evenings of flowering (Seymour and Matthews, 2006).

Thus, while changes in MR and temperature have been measured over the complete two-night flowering sequence in *V. amazonica*, it has only been partially characterised in *V. cruziana*. Furthermore, it remains to be seen whether any *Victoria* flowers possess the ability to thermoregulate, and whether the differences in heating patterns observed between *V. amazonic*a growing in the wild and *V. cruziana* growing under glasshouse conditions are also seen in plants growing under naturally fluctuating temperatures. To address this gap in our knowledge, the MR and temperature of *V. cruziana* flowers growing in an outdoor pond at Stellenbosch University Botanical Garden (33.9° S in South Africa), were measured over their two-night flowering cycle, and their responses to artificially induced changes in air temperature were recorded. This experimental approach provided the opportunity to determine whether *Victoria* flowers are theromoregulatory: capable of increasing their respiration rate and heat production in response to low ambient temperatures.

2. Materials and methods

2.1. *Cultivation of* V. cruziana

This study was undertaken on five *V. cruziana* plants growing in an open-air pond at the Stellenbosch University Botanical Garden, Stellenbosch, South Africa. These outdoor ponds were heated by circulating the water through solar heating panels. Water temperature varied depending on ambient temperature but was between 20 and 28 °C. The pond depth ranged between 70 and 90 cm. All measurements were made between Feb.–Apr. 2015.

The plants used were grown from seed originally sourced from the University of Helsinki Botanic Garden (accession number 2013-1/1), and Cambridge University Botanic Garden (accession number 2014-329), as well as crosses made between plants from Longwood Gardens (2013-157) and University of Helsinki Botanic Garden (2013-1). Plants were germinated in August 2014 by hanging seed in plastic zip-lock bags in a pond inside a greenhouse at the botanical gardens. The water was kept at approximately 26 °C by means of two 300 W aquarium heaters and exposed to natural light. No additional lighting was provided. Once the second hastate leaf appeared, seedlings were transplanted to 1.5 l pots and kept in the same pond. Pots contained loam soil with slow release fertiliser added (Haifa, Mulitcote 8 slow release fertiliser, approximately 250 ml per 50 L of soil). In early Oct. 2014 plants were moved to the outside ponds and transplanted into 80 l pots. These pots contained the same soil mentioned above. Plants started producing flower buds in early Jan. 2015 but these buds were cut off initially to encourage leaf and root growth. Plants were then allowed to flower from Feb. 2015 onwards. In total 15 flowers were measured over the course of this study, with individual flowers being recorded from only once for any floral chamber temperature or metabolic rate measurement.

2.2. *Floral chamber temperature*

To determine the normal pattern of thermogenesis displayed by flowers blooming on the outdoor pond, floral chamber and air temperatures were recorded from 6 flowers at 15 s intervals using 36-gauge T-type thermocouples connected to a Squirrel 800 data logger (Grant Instruments, Cambridge, UK). The thermocouple that recorded the temperature of the floral chamber was inserted into the flower through a small hole made through the side of the receptacle, just below the sepals. The hole was made with a 2 mm diameter flat-head screwdriver, inserted at a slight downward angle. This hole emerged at the base of the stylar processes, and allowed around 8–10 cm of the thermocouple to be threaded into the flower. A second thermocouple was positioned just above the surface of the pond, adjacent to the flower.

Please cite this article in press as: Schimpf, N.G., et al., Respiration, thermogenesis, and thermoregulation of *Victoria cruziana* flowers. Aquat. Bot. (2016), http://dx.doi.org/10.1016/j.aquabot.2016.11.014