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Floral thermogenesis of three species of *Hydnora* (Hydnoraceae) in Africa

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• *Background and Aims* Floral thermogenesis occurs in at least 12 families of ancient seed plants. Some species show very high rates of respiration through the alternative pathway, and some are thermoregulatory, with increasing respiration at decreasing ambient temperature. This study assesses the intensity and regulation of respiration in three species of African *Hydnora* that represent the Hydnoraceae, an unusual family of holoparasitic plants from arid environments.
• *Methods* Long-term respirometry (CO_2 production) and thermometry were carried out on intact flowers of *H. africana*, *H. abyssinica* and *H. esculenta* in the field, and short-term measurements were made on floral parts during the protogynous flowering sequence.
• *Key Results* For *H. africana*, there was no temperature elevation in either the osmophores or the gynoecial chamber in any phase, and mass-specific respiration rates of the flower parts were low (maximum 8·3 nmol CO_2 g^{-1} s^{-1} in osmophore tissue). Respiration tracked ambient and floral temperatures, eliminating the possibility of the inverse relationship expected in thermoregulatory flowers. *Hydnora abyssinica* flowers had higher respiration (maximum 27·5 nmol g^{-1} s^{-1} in the osmophores) and a slight elevation of osmophore temperature (maximum 2·8 °C) in the female stage. Respiration by gynoecial tissue was similar to that of osmophores in both species, but there was no measurable elevation of gynoecial chamber temperature. Gynoecial chamber temperature of *H. esculenta* could reach 3·8 °C above ambient, but there are no respiration data available. Antheral tissue respiration was maximal in the male phase (4·8 nmol g^{-1} s^{-1} in *H. africana* and 10·3 nmol g^{-1} s^{-1} in *H. abyssinica*), but it did not raise the antheral ring temperature, which showed that thermogenesis is not a by-product of pollen maturation or release.
• *Conclusions* The exceptionally low thermogenesis in *Hydnora* appears to be associated with scent production and possibly gynoecial development, but has little direct benefit to beetle pollinators.

Key words: Pollination biology, *Hydnora*, thermogenesis, respiration rate, temperature, flowers, insects.

INTRODUCTION

Significant floral thermogenesis occurs in several families of basal seed plants, including Annonaceae, Araceae, Arecaceae, Aristolochiaceae, Cycadaceae, Cyclanthaceae, Illiceaceae, Magnoliaceae, Nelumbonaceae, Nymphaeaceae, Rafflesiaceae and Schisandraceae (Thien *et al.*, 2009). Thermogenic species are found in a wide variety of habitats, including tropical and subtropical forests, Mediterranean ecosystems, temperate forests and snow-covered bogs. The flowers or inflorescences are typically protogynous, large, fleshy structures that produce copious pollen and contain a floral chamber where insects, chiefly beetles, are attracted by pungent odours (Gottsberger, 1988). Odour production and thermogenesis are often located in specialized organs called osmophores (Vogel, 1990; Endress, 1994), but some inflorescences have two heat-generating tissues with different functions (Seymour and Schultze-Motel, 1999). Some thermogenic species, including those in *Philodendron*, *Symplocarpus* and *Nelumbo*, are thermoregulatory, i.e. they are able to moderate floral temperature changes by increasing heat production as ambient temperature drops (Nagy *et al.*, 1972; Knutson, 1974; Seymour and Schultze-Motel, 1996). As part of a broad study of the diversity of thermogenic plants, this investigation examines three species of the holoparasitic genus *Hydnora*. This genus is of interest for two main reasons: it is the first representative of the Family Hydnoraceae and the first desert-adapted thermogenic species to be studied in detail. Thermogenesis has been reported in the South American *Prosopanche americana* (R.Br.) Baill. (Hydnoraceae; Cocucci and Cocucci, 1996) and from one other parasitic plant *Rhizanthes lowii* (Becarri) Harms (Rafflesiaceae; Patiño *et al.*, 2000). Coincidentally, the Hydnoraceae were once allied with Rafflesiaceae under some classifications (Cronquist, 1981; Takhtajan, 1997). The best current knowledge, however, places the Hydnoraceae in an early diverging lineage of angiosperms among the Piperales (Nickrent *et al.*, 2002).

Hydnora species are among the strangest plants in the world and have an apparent centre of diversity in southern Africa, where at least three species are recognized (Schreiber, 1968; Musselman and Visser, 1989). Due to their holoparasitic habit, the adult plants lack many morphological features typical of angiosperms, and are without roots, scales and leaves. These unusual plants completely lack chlorophyll (De La Harpe *et al.*, 1980) and form haustoria, intimate connections between the host root and the parasite rhizome, by which all nutritional requirements are met (Tennakoon *et al.*, 2007). Among *Hydnora* species, host specificity ranges from

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exclusive host–parasite relationships to quite broad host preference for either *Euphorbia* species or several genera in the Fabaceae (Musselman and Visser, 1989).

In all three species, the flowers are the only structures that emerge from the soil. They usually bloom singly, but can appear in groups of two or three, often in the shade of the host plant. The pollination biology of *Hydnora africana* includes a 'catch and release' insect-trapping mechanism (Bolin *et al.*, 2009). Flowers are putatively protogynous for 2–5 d (mean = 3 d), during which time they generate an odour resembling carrion from osmophores recessed in each tepal. Insects, mainly *Dermestes maculates* De Geer (Dermestidae), are drawn to the odour and fall into the floral chambers (Fig. 1). They can move between the androecial chamber above the anthers and the gynoecial chamber below them, but are temporarily detained by the glabrous vertical walls of the androecial chamber. After pollen is shed, the insects escape due to the textural changes in the interior walls of the androecial chamber. While little is known about the pollination biology of *H. abyssinica* and *H. esculenta*, these two species also attract insect pollinators through odours from osmophores. In contrast to *H. africana*, the osmophores of *H. abyssinica* and *H. esculenta* are situated at the tips of the tepals (Fig. 1).

This study was designed to characterize the patterns and intensity of thermogenesis throughout anthesis by measuring floral temperatures and respiration rates, to determine the individual respiratory contributions of floral parts and to assess whether the flowers are thermoregulatory.

MATERIALS AND METHODS

Thermography and respirometry

Whole flowers of *H. africana* Thunb. (Hydnoraceae) were studied at Middelpos (S27°32·740′, E17°52·882′) in the Gondwana Cañon Park, east of the Fish River Canyon, between 15 and 18 July 2008 and between 10 and 15 February 2009. Due to synonymy and taxonomic uncertainty in *H. africana sensu lato* and observed morphological and molecular variability (J. F. Bolin, unpubl. data), we emphasize that we are presenting data for *H. africana* parasitizing *Euphorbia gregaria* Marloth, restricted to southern Namibia and extreme northwestern South Africa. *Hydnora abyssinica* A. Braun was located at Holoog (S27°24·150′, E17°56·722′), in association with its host *Acacia karroo* Hayne, and was studied during 13–17 February 2009. *Hydnora esculenta* Jumelle & Perrier was studied at Berenty Preserve (S24 °59·861′, E46 °17·762′), Madagascar, during 7–9 December 2007, in association with its host, *Pithecellobium dulce* (Roxb.) Benth.

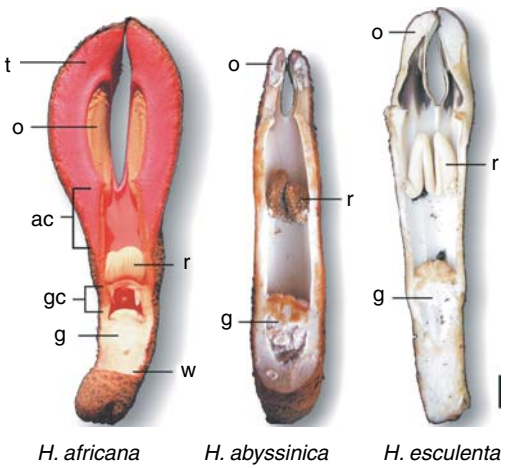

FIG. 1. Flowers of *Hydnora africana*, *H. abyssinica* and *H. esculenta* largely share the same basic plan. However, the osmophores (o) of *H. africana* are recessed on the interior surface of each tepal (t) but are apical in *H. abyssinica* and *H. esculenta*. The *Hydnora* chamber flower is comprised of androecial (ac) and gynoecial (gc) chambers divided by an antheral ring (r). The stigma is positioned on the floor of the gynoecial chamber and subtended by the gynoecial tissue (g). The floral wall (w) surrounds the gynoecial chamber and gynoecial tissue, and includes the base of the flower. Scale bar = 2 cm.

Respirometry of flowers was carried out in the field with both long-term and short-term measurements. For long-term studies, flowers were partially excavated down to the level of the gynoecial chamber, and the soil surface around the flower was sealed with two layers of cling wrap to prevent sampling of CO_2 derived from soil. A hood fashioned from a plastic 300 mL drink bottle with the bottom cut off was placed over the flower and partly sealed with layers of cling wrap around the base. The open-flow respirometry system was essentially the same as described in detail earlier (Seymour and Gibernau, 2008). Air was pumped from the chamber with a constant flow air sampling pump (Gil-air Model 3; www.sensidyne.com) at about 400–600 mL min^{-1} as measured with mass flowmeters (model 822, www.sierrainstruments.com), calibrated with a bubble flowmeter (Gilibrator, www.sensidyne.com) into a CO_2 analyser (Li-Cor model 820, www.licor.com). The output of the analyser was recorded at 2 min intervals with a data logger (series 1203, www.grant.co.uk), along with temperature data from T-type thermocouples placed in the gynoecial chamber, the osmophore tissue on the inside of the tepal, the air in the respirometry hood, and the ground, adjacent to the flower and at gynoecial level. The hood and flower were shaded from solar radiation throughout the recording period with a cardboard box, skewered to the ground and open on the south side for ventilation.

Short-term measurements were carried out on shaded flowers cut at selected stages in the field. They were severed from the plant, recut with a wet knife and the cut immediately placed in shallow water until measurements were complete, within 2 h. The respirometry system was the same as described above, except for the chamber that had an adjustable volume. Parts were cut sequentially from the flower, placed in the chamber and measured after equilibrium (about 4 min at a flow of 400 mL min^{-1}). Tissue temperatures were taken with a needle thermocouple attached to a thermometer (model 52, www.fluke.com). The parts were placed in sealed plastic bags and weighed within 2 h with a digital balance (model 1210-100, Tanita Corporation, Tokyo, Japan). The order of measurement was: osmophores, tepals (down to the antheral ring), antheral ring, gynoecium (stigma with ovary) and floral walls (see Fig. 1). Thus the entire flower was measured. In some cases, both osmophores and tepals were measured together, followed by osmophores separately, to obtain tepal respiration by subtraction. Tissue types were clearly

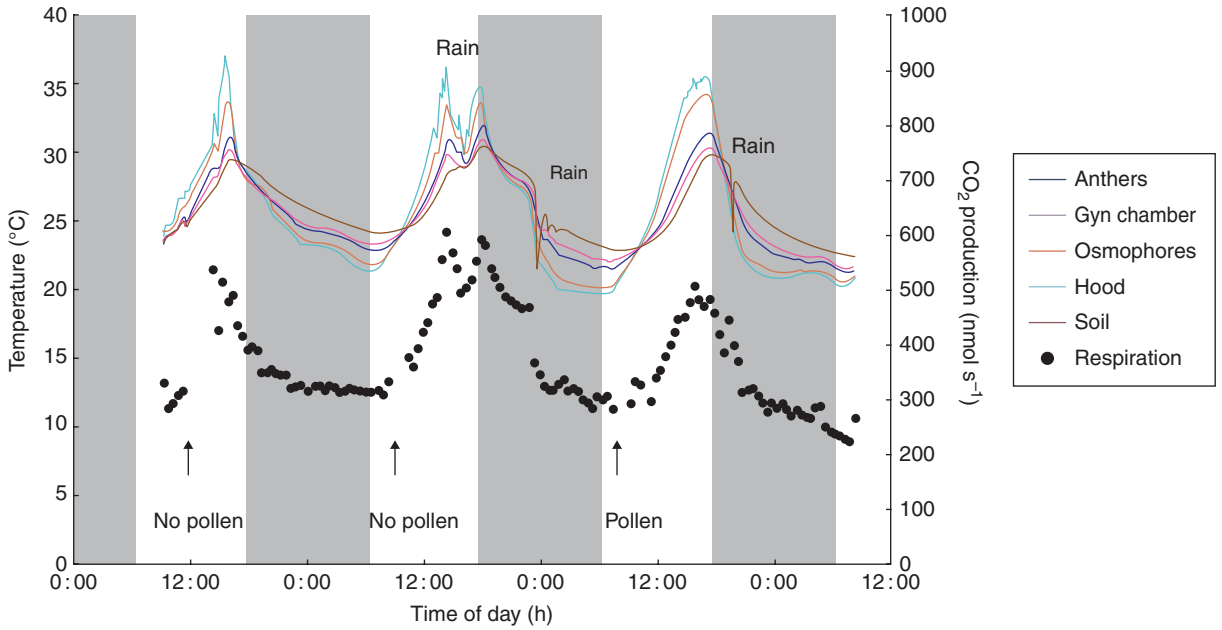

FIG. 2. Temperatures and respiration rate of a *H. africana* flower over three days in summer. Night is shaded. Temperatures were recorded in the antheral ring, the gynoecial chamber, one osmophore in a tepal, the air in the respirometry hood and the soil adjacent to the gynoecial chamber. Respiration rate was recorded from the entire flower. The record began in a freshly opened flower in the female phase, when the osmophores were releasing scent, and continued through the male phase that began when the pollen was released at some time during the second day. Presence or absence of pollen is indicated at times when the flower was observed. Steep decreases in hood and soil temperatures were associated with rain showers.

distinguishable. Although the osmophores of *H. africana* are surrounded by tepal tissue, there was a clear layer of darker tissue that separated them.

Thermography of *H. esculenta* was carried out on two flowers in the female stage using data loggers (Hobo model H12 with K-type, 5 mm thermocouples; www.onsetcomp.com). The flowers were excavated to the level of the ovary. One thermocouple was then placed in the gynoecial chamber, touching the stigma, and another was placed in air outside of the chamber. Logging continued for approx. 23 h, during which time the flowers were shaded from the sun.

Statistics are means and 95 % confidence intervals (CIs) for the mean. Student's *t*-test was used to find significant differences at the 0·05 % level. Least squares regressions were fitted to data in Microsoft Excel.

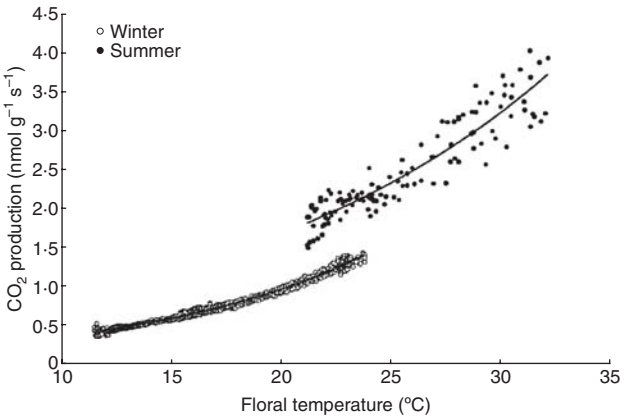

FIG. 3. Relationships between mass-specific respiration rate and mean floral temperature of two whole *H. africana* flowers in winter and summer. The exponential equations relating respiration rate (M_{CO_2}) and mean floral temperature (T) are $M_{CO_2} = 0{\cdot}1278e^{0{\cdot}1007T}$ ($R^2 = 0{\cdot}99$) and $M_{CO_2} = 0{\cdot}4448e^{0{\cdot}0663T}$ ($R^2 = 0{\cdot}87$), respectively.

RESULTS

Long-term thermometry and respirometry

Hydnora africana. The longevity of the protogynous *H. africana* flower is variable, with the female phase averaging 3 d when insects are attracted, followed by the 1 d male phase when pollen is shed over several hours, after which trapped insects begin to escape (Bolin *et al.*, 2009). Recordings over 3 d were undertaken on two *H. africana* flowers, one in July 2008 (winter) and another in February 2009 (summer; Fig. 2). Both recordings were begun in the early female phase, when the flowers had just opened. The patterns were similar in both, although ambient temperature in the respirometry hood varied between about 5 and 25 °C in winter and from 20 to 37 °C in summer. There was no obvious elevation of osmophore temperature at any time, even during initial opening, when the flower emitted the carrion-like odour; the temperatures were always within about 1 °C of hood temperatures, lower in the day and higher at night. These differences were interpreted as thermal lags, as the temperatures deeper in the flower showed greater buffering from the environmental extremes. There were also no apparent elevations in antheral ring temperatures associated with pollen shedding. Floral respiration rate paralleled floral temperatures, including sharp drops associated with cooling rain periods (Fig. 2).

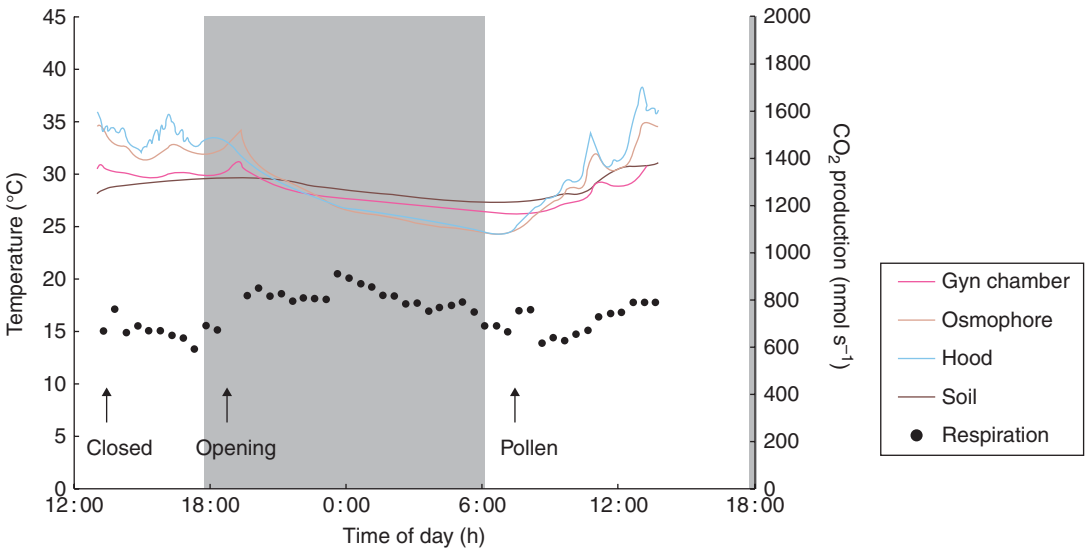

FIG. 4. Continuous records of environmental and floral temperatures and respiration rate during blooming of *Hydnora abyssinica*. Night is shaded. Times of spot observations are indicated.

When all of the data for mass-specific respiration and mean floral temperature from long-term measurements were regressed, the direct temperature effect on respiration became evident (Fig. 3). Exponential equations fitted to the data indicated the factor that a 10 °C rise in temperature increased the rate (Q_{10}), with values of 2·74 (winter) and 1·94 (summer).

Hydnora abyssinica. The sequence of flowering in *H. abyssinica* finished within 24 h, which was much faster than the several days required by *H. africana*. Four flowers were recorded from the female phase through pollen shedding, and the population was observed at least three times each day, in the morning (approx. 0730 h), at mid-day (approx. 1300 h) and in the evening (approx. 1830 h). Opening could occur at unpredictable times, either during the day, before 1600 h, or during the night, after 1900 h. Only one flower was observed opening, and this occurred around 1830 h (Fig. 4). Of the four flowers recorded continuously, three exhibited slight warming of the osmophores above hood temperature. In two cases, the maximum excess temperatures were 2·0 and 2·3 °C, and occurred when the flower was opening (Fig. 4). In the third case, the maximum of 2·8 °C occurred after all of the pollen was shed. The fourth flower showed no rise. The rate of oxygen consumption in all four flowers was positively related to floral temperature, with a collective Q_{10} of 1·87.

Hydnora esculenta. Temperature records were opportunistically available for two flowers in the female stage, obtained on successive days. Both showed similar patterns of stable or slightly rising gynoecial temperatures as ambient temperature fell during the night (Fig. 5). The maximum temperature difference was 3·8 °C at 0440 h in one and 1·7 °C at 0400 h in the other.

Short-term respirometry

Hydnora africana. Because the exact durations of floral stages were not known, flowers were classified in three

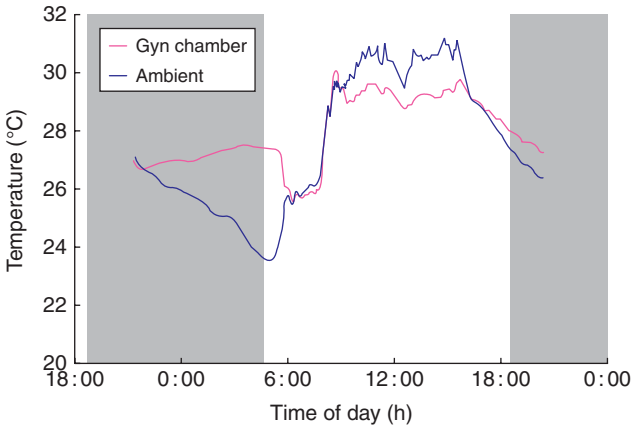

FIG. 5. Temperatures in the gynoecial chamber and adjacent ambient air in *H. esculenta* during the female phase of blooming. Night is shaded.

stages – bud, female and male stages – as determined by opening and shedding of pollen. We could also estimate whether the stage was early or late from the appearance of the flower. Flowers in early female stages showed creamy white osmophores in succulent tepals, while older ones had browning osmophores in thinning tepals. The early male stage was judged by incompletely or freshly shed pollen, while the later stage was characterized by wilting tepals and thin, leathery osmophores. Because the measurements were made at uncontrolled temperatures in the field, the rates were corrected according to the van 't Hoff/Arrhenius effect. In the winter of 2008, temperatures of short-term measurement samples ranged from 15·1 to 25·4 °C, but in summer of 2009 the range was 21·4–32·9 °C. Data were therefore corrected to a common, moderate temperature of 25 °C by applying an exponential temperature dependence curve derived from long-term measured flowers at the time (Fig. 3).

The fleshy tepals and floral walls had the highest total respiration rates, because they comprised the largest mass

TABLE 1. *Rate of CO_2 production ($\dot{M}_{CO_2}$) of floral parts of* H. africana *at selected stages of development*

Stage	Part	Mass (g)	$\dot{M}_{CO_2}$ (nmol s^{-1})	$\dot{M}_{CO_2}$ (nmol g^{-1} s^{-1})	$\dot{M}_{CO_2}$ (fraction)
Bud $n = 5$	Osmophore	5·2 (2·3)	30 (9)	6·3 (1·4)	0·11 (0·02)
	Tepal	73·4 (21·6)	131 (42)	1·9 (0·7)	0·48 (0·09)
	Antheral ring	4·5 (1·2)	9 (5)	2·1 (0·7)	0·04 (0·02)
	Gynoecium	3·4 (0·8)	33 (7)	10·0 (2·7)	0·13 (0·03)
	Floral wall	20·0 (7·5)	63 (16)	3·4 (0·8)	0·24 (0·07)
	Total	106·5 (31·4)	267 (41)	2·7 (0·7)	1·00
Female 1 $n = 5$	Osmophore	8·5 (2·9)	70 (30)	8·3 (2·9)	0·19 (0·03)
	Tepal	93·7 (26·6)	148 (52)	1·6 (0·2)	0·43 (0·07)
	Antheral ring	5·0 (1·5)	9 (2)	2·1 (1·1)	0·03 (0·02)
	Gynoecium	4·5 (1·0)	37 (18)	8·2 (2·9)	0·11 (0·03)
	Floral wall	27·8 (10·9)	83 (31)	3·1 (0·6)	0·24 (0·03)
	Total	139·4 (39·6)	347 (110)	2·5 (0·4)	1·00
Female 2 $n = 5$	Osmophore	3·8 (0·9)	23 (12)	6·0 (2·8)	0·09 (0·05)
	Tepal	55·0 (12·0)	134 (49)	2·4 (0·4)	0·48 (0·14)
	Antheral ring	4·3 (0·8)	10 (4)	2·2 (0·6)	0·03 (0·01)
	Gynoecium	3·7 (1·4)	43 (23)	11·1 (1·7)	0·14 (0·02)
	Floral wall	33·8 (35·8)	106 (105)	3·3 (0·9)	0·27 (0·14)
	Total	100·6 (38·8)	315 (150)	3·0 (0·4)	1·00
Male 1 $n = 5$	Osmophore	2·0 (0·4)	15 (6)	8·0 (4·6)	0·06 (0·02)
	Tepal	52·8 (11·8)	132 (45)	2·5 (0·5)	0·51 (0·03)
	Antheral ring	2·9 (0·8)	11 (3)	4·3 (1·9)	0·05 (0·01)
	Gynoecium	3·7 (1·1)	36 (14)	9·7 (2·4)	0·14 (0·03)
	Floral wall	18·6 (8·3)	59 (16)	3·6 (1·1)	0·24 (0·02)
	Total	79·9 (19·0)	253 (75)	3·2 (0·6)	1·00
Male 2 $n = 5$	Osmophore	3·2 (1·4)	15 (13)	4·7 (3·1)	0·05 (0·03)
	Tepal	51·0 (22·2)	113 (47)	2·2 (0·4)	0·40 (0·09)
	Antheral ring	3·2 (1·3)	14 (3)	4·8 (1·6)	0·05 (0·02)
	Gynoecium	4·6 (1·1)	49 (12)	11·0 (2·6)	0·19 (0·08)
	Floral wall	22·3 (10·3)	79 (15)	4·1 (1·4)	0·30 (0·04)
	Total	84·3 (34·1)	269 (66)	3·4 (0·5)	1·00

Female and male stages are divided into early (1) and late (2) parts.
Values are means with 95 % confidence intervals in parentheses.

(Table 1); their respiration rates did not change significantly during blooming (Fig. 6A). The osmophore tissues showed appreciable respiration, and were significantly higher in the early female phase, when the flower was most odiferous (Fig. 6A). Respiration of the gynoecial tissue was also moderate. Respiration of the antheral ring was the lowest, but with a statistically insignificant tendency to increase in the male phase. Respiration of all of the parts in the early female stage averaged 347 ± 110 (CI) nmol s^{-1} (Table 1), which is not significantly different from the average of 268 ± 91 nmol s^{-1} taken from two long-term flowers in the female stage when floral temperature was about 25 °C.

On a mass-specific basis, the respiration rate was highest in the osmophores and gynoecial tissues of *H. africana* (Fig. 6B). There were no significant changes in the values of any of the tissues throughout the flowering sequence, except in the antheral ring that increased in the male phase. The changes in total respiration of the osmophores during the early female phase (Fig. 6A) were due simply to changes in the mass of the osmophore tissue (Table 1).

Hydnora abyssinica. The stages of *H. abyssinica* were categorized into bud, female, transitional and male stages, because the stages were short and did not need dividing. The new stage 'transitional' was given to flowers that had partially exserted pollen. The respiration of the whole flower was maximal in the female and transitional stages (Table 2). In the female stage, the osmophore accounted for 45 % of the total respiration, but decreased significantly thereafter. On a mass-specific basis, the respiration of the osmophore was the highest, especially in the female stage (Fig. 7). There was a significant increase in respiration of the antheral ring between the female and male phases, on both a total and a mass-specific basis (Fig. 7, Table 2).

We have spot measurements for two flowers in the female stage, and unfortunately they gave greatly different values for osmophore respiration (Table 2). However, this result is consistent with the observation that the osmophore temperature could either be similar to hood temperature or a few degrees above it during the female phase in long-term measurements. Overall, the mass-specific respiration of osmophore tissue in the female phase was about three times higher in *H. abyssinica* (Fig. 7) than in *H. africana* (Fig. 6B).

Respiration of all of the parts in the female stage averaged 658 ± 180 (CI) nmol s^{-1} (Table 2), which is not significantly different from the average of 636 ± 170 nmol s^{-1} taken from four long-term flowers in the female stage when floral temperature was about 25 °C.

Insects found in Hydnora

Dermestid beetles (*Dermestes maculatus*) weighing on average 28 mg were found in *H. africana*. Many unidentified scarab beetles (10–104 mg) and one tenebrionid mouldy beetle (*Eurychora* sp.) (170 mg) were present in

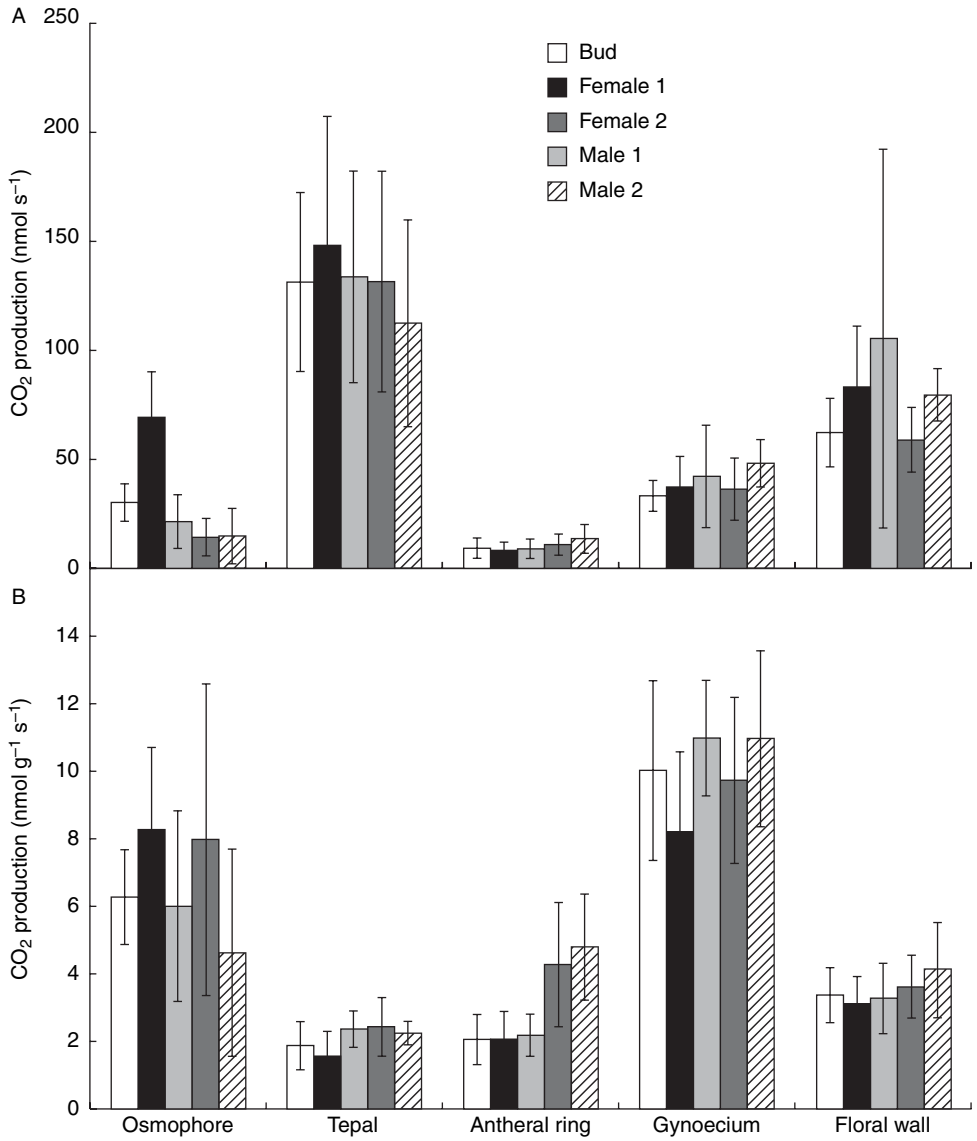

FIG. 6. Respiration rate of whole floral parts (A) and mass-specific tissues (B) of *H. africana* during the flowering sequence. All data are adjusted to a constant temperature of 25 °C (see text). Five classes of flower stage are represented in order, from bud, through two stages of female and two stages of male. Error bars are 95 % confidence intervals of the means from five flowers. The high variability evident in the floral wall of the late female phase is due to a single flower that had an exceptionally long floral base.

H. abyssinica. Several 6 mm long, unweighed and unidentified scarabs were observed within *H. esculenta*.

DISCUSSION

Thermogenesis

This study was aimed at assessing the extent and magnitude of metabolic thermogenesis in species of *Hydnora*. It is clear that the flowers were thermogenic, but the phenomenon differed between the three species. In *H. africana*, thermogenesis occurred throughout the female and male phases (Fig. 2) but, when corrected to a common temperature to eliminate external thermal effects, the intensity was marginally greater in the female phase, when the flower was producing the most scent (Table 1). In this species, the osmophores accounted for only 19 % of total heat production of the flower in the female phase, and less in the other phases (Table 1). The osmophores of *H. abyssinica* were more thermogenic, being responsible for 45 % of the total heat production in the female phase (Table 2) and clearly associated with scent production. Nevertheless, the relative contributions of the scent-producing organs were considerably less than in other thermogenic species (Nagy *et al.*, 1972; Seymour and Schultze-Motel, 1998; Seymour and Schultze-Motel, 1999; Seymour and Matthews, 2006). Part of the reason for the low contribution by the osmophores was a relatively large amount of structural tissue in the tepals and floral walls. Although the mass-specific respiration values of these structural tissues were very low

TABLE 2. *Rate of CO_2 production ($\dot{M}CO_2$) of floral parts of* H abyssinica *at selected stages of development.*

Stage	Part	Mass (g)	$\dot{M}CO_2$ (nmol s^{-1})	$\dot{M}CO_2$ (nmol g^{-1} s^{-1})	$\dot{M}CO_2$ (fraction)
Bud $n = 1$	Osmophore	12·4	66	5·4	0·19
	Tepal	25·9	75	2·9	0·21
	Antheral ring	4·7	20	4·1	0·05
	Gynoecium	5·9	29	4·9	0·08
	Floral wall	50·5	168	3·3	0·47
	Total	99·4	357	3·6	1·00
Female $n = 2$	Osmophore	11·4 (1·3)	307 (222)	27·5 (22·6)	0·45 (0·21)
	Tepal	38·1 (4·1)	163 (41)	4·3 (1·6)	0·26 (0·13)
	Antheral ring	5·9 (0·2)	19 (0)	3·3 (0·1)	0·03 (0·01)
	Gynoecium	5·4 (0·2)	25 (2)	4·7 (0·3)	0·04 (0·01)
	Floral wall	76·8 (9·0)	144 (1)	1·9 (0·2)	0·22 (0·06)
	Total	137·6 (11·4)	658 (180)	4·8 (0·9)	1·00
Transitional $n = 3$	Osmophore	8·1 (0·7)	131 (60)	14·6 (4·8)	0·23 (0·08)
	Tepal	38·7 (7·4)	138 (4)	3·6 (0·7)	0·25 (0·03)
	Antheral ring	5·1 (0·2)	49 (0)	10·3 (0·3)	0·09 (0·00)
	Gynoecium	7·1 (0·0)	40 (4)	5·6 (0·6)	0·07 (0·01)
	Floral wall	76·1 (28·2)	189 (10)	2·8 (0·8)	0·35 (0·04)
	Total	134·9 (36·2)	547 (42)	4·2 (0·6)	1·00
Male $n = 4$	Osmophore	7·6 (3·0)	134 (52)	16·2 (5·6)	0·20 (0·11)
	Tepal	31·3 (1·1)	189 (49)	6·2 (1·9)	0·33 (0·14)
	Antheral ring	5·4 (2·6)	47 (24)	8·8 (0·9)	0·08 (0·05)
	Gynoecium	8·0 (3·3)	36 (14)	4·4 (0·4)	0·06 (0·03)
	Floral wall	61·5 (7·7)	185 (9)	3·1 (0·6)	0·33 (0·05)
	Total	113·7 (17·6)	593 (31)	5·2 (0·4)	1·00

Values are means with 95 % confidence intervals in parentheses.

(Figs 6B and 7), their cumulative masses elevated their contributions to the total.

Thermogenesis was so weak in these species that there was very little elevation of floral temperatures. In *H. africana*, there was no measurable increase in osmophore temperature over ambient temperature (Fig. 2), and the elevation in *H. abyssinica* was only about 2 °C upon opening (Fig. 4). However, temperature measurements alone are not good indications of thermogenesis, especially at high ambient temperatures, because evaporative heat loss can offset respiratory heat production, sometimes leading to no temperature elevation or even temperature depression (Seymour and Schultze-Motel, 1998; Seymour and Schultze-Motel, 1999). In the present case, however, the measured low thermogenesis seems largely responsible for the lack of temperature rise, although we did not measure the evaporation rate.

On a mass-specific basis and corrected to 25 °C, the metabolic intensity of *Hydnora* was relatively weak. *Hydnora africana* osmophores reached about 8 nmol g^{-1} s^{-1} (Fig. 6B), and *H. abyssinica* reached 28 nmol g^{-1} s^{-1} (Fig. 7), in the female phase. By comparison, the spadix of skunk cabbage *Symplocarpus foetidus* respires at 105 nmol g^{-1} s^{-1} at 25 °C, where tissue and ambient temperatures are identical (Seymour, 2004). Maximum mass-specific respiration rates of other thermogenic flowers can reach 360 nmol g^{-1} s^{-1} in *Philodendron selloum* (Nagy *et al.*, 1972), 450 nmol g^{-1} s^{-1} in *Arum maculatum* (Lance, 1974), 820 nmol g^{-1} s^{-1} in *Helicodiceros muscivorus* (Seymour *et al.*, 2003*a*) and 920 nmol g^{-1} s^{-1} in *Arum concinnatum* (Seymour *et al.*, 2009*a*).

Temperature regulation was not evident in *H. africana* or *H. abyssinica*. A regulatory response is indicated either by a degree of independence of floral temperature from ambient temperature or by a reversible, inverse relationship between respiration rate and tissue temperature (Seymour, 2001). Respirometry is preferable, because it is not subject to external influences such as evaporation or radiation. Neither measurement indicated temperature regulation. Floral temperature generally followed ambient temperatures through the daily cycles (Figs 2 and 4). Respiration increased, rather than decreased, with tissue temperature and followed a van 't Hoff/Arrhenius relationship with a Q_{10} of approx. 2 (Fig. 3). These results partially accounted for the lower respiration rates of *H. africana* in winter compared with summer at common temperatures (Fig. 3). If this species were thermoregulatory, one would expect higher respiration in winter. The failure of winter and summer data to coincide at common temperatures cannot be explained, except to point out that they represent only two individuals that may have had different inherent mass-specific respiration rates. Such variability was evident in short-term measurements of this species (Fig. 6B). However, temperature measurements of *H. esculenta* were suggestive of regulatory ability, because gynoecial temperature was somewhat independent of decreasing ambient temperatures during the night (Fig. 5). Unfortunately we have no respirometric data to determine whether the temperature differences resulted from augmented heat production. Increasing humidity during the night could have reduced evaporative heat loss and raised floral temperature. Moreover, experimental manipulation of ambient temperature would be required to determine whether the response is true, reversible regulation or a 'pseudo-thermoregulatory' pattern that heats the flower at night, in response to light cycle, not low temperature (Seymour *et al.*, 2003*a*).

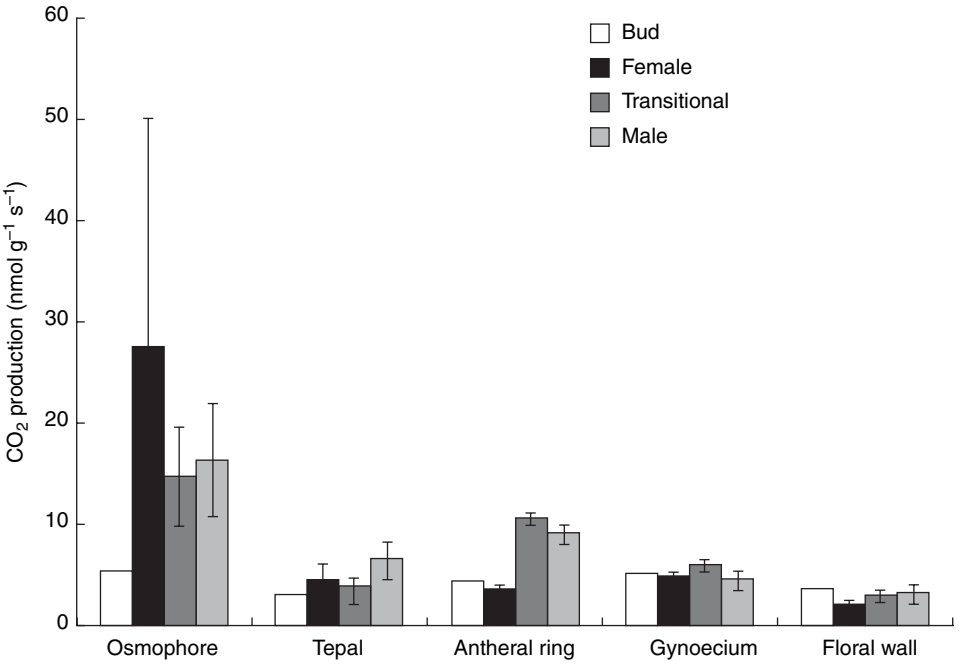

FIG. 7. Mass-specific respiration rate of *H. abyssinica* floral parts, adjusted to 25 °C. Flowering stages are bud, female, transitional and male. Data are means and 95 % confidence intervals from six flowers.

It is significant that the mass-specific respiration of the antheral rings was generally low compared with the other structures, although it did increase slightly between the female and male phases in both species (Figs 6B and 7). Nevertheless there was no increase in antheral ring temperature in *H. africana* (Fig. 2). Intense thermogenesis occurs in the male florets of arum lilies (Seymour *et al.*, 1983; Seymour and Schultze-Motel, 1999), in the stamens of the sacred lotus *Nelumbo nucifera* (Seymour and Schultze-Motel, 1998) and *Victoria amazonica* (Seymour and Matthews, 2006), and in the sporophylls of male cycad cones (Seymour *et al.*, 2004). It has thus been reasonable to propose that the maturation and release of pollen are associated with thermogenesis, possibly as a metabolic by-product (Gibernau *et al.*, 2004). However, a functional relationship has never been demonstrated in any thermogenic species, and *Hydnora* clearly demonstrate that copious pollen can be produced without intense respiration. It becomes clear that thermogenesis in the male structures of other groups is probably related to some function other than pollen production. It is also interesting that the mass-specific respiration rates of the gynoecial tissues were similar to those of the osmophores in *H. africana* (Fig. 6B), however somewhat lower in *H. abyssinica* (Fig. 7). The role of this respiration is not known, but may be related to the growth and development of the ovules.

The data from *H. africana* and *H. abyssinica* are at variance with temperature data from *H. esculenta* and a related South American member of the Hydnoraceae, *P. americana*. The gynoecial chamber of *H. esculenta* was up to 3·7 °C above ambient (this study) and the antheral body of *P. americana* was reported to be up to 6 °C above ambient in the evening (Cocucci and Cocucci, 1996). These differences may be correlated with the phylogenetic history of the family. A two-gene phylogeny of the Hydnoraceae suggests close affinities between the Fabaceae-parasitizing species, *Prosopanche* spp., *H. esculenta* and *H. abyssinica*, which appear to be more thermogenic than the more derived *H. africana* complex (J. F. Bolin, unpubl. data).

Ecological implications

The ecological role of thermogenesis in flowers is generally thought to be associated primarily with enhancing the volatilization of insect-attracting floral scents (Fægri and van der Pijl, 1979; Meeuse and Raskin, 1988; Endress, 1994). Other functions have been demonstrated, including a thermal reward to adult insect visitors (Seymour *et al.*, 2003*b*), enhancement of fertilization (Li and Huang, 2009) and optimization of pollen tube function (Seymour *et al.*, 2009*b*). Still other functions have been postulated, such as protection from freezing (Knutson, 1974) and provision of suitable environments for larval insect development (Ervik and Barfod, 1999). However, the main role of thermogenesis in *H. abyssinica* and *H. africana* is apparently scent production; because there is little chance of freezing, thermogenesis is not intense enough to raise floral chamber temperature significantly, and there is no evidence that carrion larvae develop in the floral tissues (Bolin *et al.*, 2009). Although respiration is measurable in the gynoecial tissue, which forms the bottom of the gynoecial chamber, temperatures in the chamber are scarcely different from ambient in *H. abyssinica* and *H. africana* (Figs 2 and 4). Even though the floral chamber of *H. esculenta* is warmed, the absolute temperature rise is slight (Fig. 5). Thus the insects

that reside in the chambers during the female phase do not benefit much from thermogenesis. It might be argued that flowering in the warm daytime temperatures of arid Namibia and southern Madagascar has diminished the value of thermogenesis; however, the present study shows that the gynoecial chamber temperature of *H. africana* can drop to 21 °C in summer and to 11 °C in winter. These temperatures are much below the preferred activity temperatures of most beetles, which are generally above 30 °C (Heinrich, 1993). This may relate to the size of beetle pollinators of *Hydnora*. Our opportunistic records indicated that most beetles weighed <100 mg. Although higher temperature may enhance the activity of insects of all sizes (Heinrich, 1993), high temperatures yield substantial energy savings only in endothermic beetles that are able to raise their own body temperatures by metabolic heat production (Seymour *et al.*, 2003*b*), and this is increasingly rare in smaller species (Oertli, 1989). The situation may be similar in *P. americana*, which permits only small nitidulids into the gynoecial chamber (Cocucci and Cocucci, 1996). Alternatively, deception trap flowers may not be obliged to offer any reward to insects fooled into visiting them. Indeed, it might be valuable for trapped insects to remain cooler and save energy during their imprisonment that can last several days in *H. africana.*

Conclusions

Although the three species of *Hydnora* that we studied are thermogenic, they are clearly at the lower end of the spectrum of heat-producing plants, and they are not thermoregulatory. This may be associated with their unusual life history, including a parasitic, sub-terranean existence in arid environments and attraction of small insects that might not require high temperatures. Because this is the first study on plants in the Family Hydnoraceae, and there are no other species that resemble their natural history, however, we may never fully understand the role of thermogenesis in this group.

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