SOME THERMAL RELATIONSHIPS OF TROPICAL FLOWERS

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A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF EDINBURGH

2001

Dedication

I dedicated this thesis to my family and friends that are not here but always have been with me.

Abstract

A unified approach was used to analyse the energy budgets and temperatures of flowers belonging to the families *Rafflesiaceae* and *Convolvulaceae*; and some broader ecological and evolutionary implications of their thermal regimes were discussed.

Rhizanthes lowii and *Raflessia tuan-mudae* (*Rafflesiaceae*) are rare parasitic plants adapted to live in the understory of the tropical rain forest in South East Asia. In the understory of the forest the ambient conditions are nearly constant (high relative humidity, low incident radiation and relatively constant air and soil temperature). These plants are parasitic on the vine *Tetrastigma*. They lack leaves, stems, or photosynthetic tissue and are characterised by gaseous emissions that attract the natural pollinators, carrion flies.

The internal and surface temperatures of the flowers were continuously monitored with fine thermocouples in different parts of the flowers whilst radiation fluxes and microclimatic variables were recorded. In the case of *Rhizanthes lowii* there was evidence of both thermogenesis and thermoregulation. Endothermy was detected in young and mature buds as well as in blooming flowers and even in decaying tissues three or more days after blooming. Tissue temperatures were maintained at 7 - 9 K above air temperature. In *Rafflesia tuan-mudae* it was found that the internal parts of the flower were maintained a few degrees (1-6 K) above air temperature and the maximum heating was in the evening.

As they are parasitic, they have the advantage over most other species as the respiratory substrate is derived from the host plant. High concentrations of CO_2 may be released as the product of respiration. A fluid dynamics model was used to estimate the CO_2 concentration in the reproductive cavity of *Rafflesia tuan-mudae*. The model suggested that the flower developed high concentrations of carbon dioxide inside the diaphragm, mainly around the central column where the cavity with the reproductive parts (anther or stigma) are located.

In both species the heating of the flower appears to assist the emission of volatile compounds, and to maintain a high concentration of carbon dioxide due to respiration. Thus, heat, volatiles and CO_2 seem to be components in the mimicry of rotten flesh or infected wound tissue to attract the pollinating blowflies.

In contrast to the cases mentioned above, *Ipomoea pes-caprae*, *Ipomoea aquatica* and *Merremia borneensis* (*Convolvulaceae*) are generally numerous, short lived, and produced in sunny situations including beaches and along the sides of roads. They attract pollinators by being visually conspicuous; but to achieve this they must face the sun.

The temperature of these flowers was studied in bright sunshine, to determine the role of corollas and sepals in cooling the gynoecium. Corollas and sepals were prevented from transpiring, by greasing, to investigate the effect of evaporation on cooling. To determine the effect of temperature on flower tissue, pollen viability and pollen germination flowers of *Ipomoea pes-caprae* and *Merremia borneensis* were exposed to different temperatures.

Manipulative experiments demonstrated that the characteristic heliotropic trumpet-shape of the convolvulaceous corolla is not merely an advertisement to attract insects but also a parasol or radiation shield to avoid overheating of the gynoecium.

Acknowledgements

This thesis would not be possible without many people that directly or indirectly contributed to this happy end. I will try to name all those that in one way or another helped me to make it possible to complete my thesis. I apologise if I miss any of you.

First of all I wish to thank Professor John Grace, my supervisor, for his guidance and good will to listen and discuss; for his insight, he knew what I was talking about; for all his help, support, patience, and good humour which contributed to make the period of studies a very enjoyable and rich personal and intellectual experience. *Thank you.*

I would like to thank Dr. Cristian Samper K. Director of the Instituto de Investigaciones Biológicas Alexander von Humbolt. Financial assistance for my studies was possible through a COLCIENCIAS scholarship. Financial support for my field work in Brunei and Sarawak was made available from the Davies Expedition Fund and from the Development Trust of the University of Edinburgh.

To my family specially my mother Ofelia, for all her love and support. To Carlos Julio and Beatriz for always being with me.

I would like to thank Dr. Chris Jeffree for always having the right formula. Thanks to: Yadvinder Malhi for his help, bringing good ideas for my first writing and for being always kind, to all my colleagues, specially to James Irvine for his technical support, Craig Barton, Peter Levy, Patrick Meir, Fiona Carswell, Alisdair Hardie, Lisa Wingate, Caroline Nichol, Johanna Pulli and Brian McIntosh. I wish to thank Robert Clement for his technical support and for writing a program for my statistical analysis. To Mark Rayment, Natalia Gomez and Mike Perks, for their friendly support. To Barbara Vinceti for being my special friend even when I started to get mad at the end of my writing, and other friends Simone

Testa, Anna Efstathiou, Maddalena Scarlino, Massimo Quinto, Jose Arevalo, Sara Strati, Juan Busque, and Caroline Wood. And many people from the Institute: Connie Fox for always finding the right thing to say and because every letter she wrote for me was successful; Shiela Wilson, always helping me with a smile; Derek Scott who always found what I needed, Malcolm Ritchie, Graham Walker and Andy Gray; Tom Whitelaw, Joe Mushet and David Waterson the servitors. In the workshop: Eric Larsen, Davie Mackenzie, George Steedman, and Alex. In the photo-lab, special mention to John Anthony for his advice and help with all my photographic work; Dave Haswell; Sir Frank Jonhston for being so special, and Graham Brown. In the glasshouse: Bob Astles and Billy Adams; Allister Kydd for his magic hands on computers.

At the Universiti Brunei Darussalam, Dr. David Edwards, Dr. Joseph Charles, and special thanks to Dr. Peter F. Becker for stimulating discussion and encouragement, and for helping me with the insect observations on the beach. I also thank his students Iryani BT Tarip, Sim Yu Hock, Hjh Siti Fatimah, Muhamad Jusran, Chany Yem En, Ang Bee B. and Albert. For this job I also thank Beth Crase, Emmanuelle Jousselin, Laure Grison, Eliane Grison and Kun-famg Cao. To Webber Booth for his help with the measurements of petals, and to his family Aida, Marcilea, Lindcea and Oddy, Ebony, the turtles, the fifty chickens, the fig tree and many other marvellous things in his garden. In the Chemistry Department, I thank Dr. Alice Edwards for doing the GC-MS analysis of the volatiles and for making our work very fun and enjoyable. To my friends Helen Pang Yoke Kiew, Awg Samhan b Nyawa, Dr. Colin Maycock and all the technicians in the Biology Department. I thank the staff at Kuala Belalong Field Studies Centre, Hjh Masnah HJ. Mirasan, Rodzay bin HJ. Abd Wahad, Ramlah, Gawin, Chibto, Nor, Sam, Nalam, Wan, Enchu, Kuni, Muja, Annie and Kingkong. Without them life in Belalong could not have been such fun.

From Sarawak I thank Mr. Ernest Chai, and the staff at Taman Negara Gunung Gading: Mr. Sem Pasam, Jamel, specially I thank John Reky and Isah. And to the memory of Batien for his friendly help and the adventures in the forest when we went to search for *Rafflesia* flowers.

From Sabah I thank Dr. Jamili Nais from Sabah Parks.

In Helsinki: Dr. Tuula Aalto and Dr. Timo Vesala helped me with the calculations of heat transfer.

Finally, I especially thank Dr. Martine Hossaert-McKey for teaching and helping me with the volatile collections from the Rafflesia flowers, Dr. Doyle McKey, Professor Melvin Tyree, Dr. John McIntyre, Dr. Hans Banziger, Dr. Chabtai Cohen, Dr. Micheal M. Blanke, Marlis and Dennis Merbach and Professor Ulrich Maschwitz.

Did I forget anyone?

Symbols and Abbreviations

Greek Alphabet

δ	solar declination (degrees)
β	azimuth angle of the plant surface (degrees)
$\propto$	azimuth angle of the sun (degrees)
α	coefficient of thermal expansion of air at 25 °C (1/298 K^{-1}) or fraction of absorbed irradiation (dimensionless)
μ	coefficient of viscosity or dynamic viscosity (N s m^{-2})
ρ	density of dry air (1.183 kg m^{-3}). *Subscript*: ρ_t of plant tissue (700 kg m^{-3}) or fraction of reflected irradiation (dimensionless)
ε	emissivity of the plant surface, assumed to be 0.96 (dimensionless)
ν	kinematic viscosity coefficient of air (1.55×10^{-5} m^2 s^{-1})
λ	latent heat of vaporisation of water (2.430×10^6 J kg^{-1} at 30 °C)
φ	latitude (degrees)
γ	psychrometer constant (66.6 Pa K^{-1})
π	ratio of circumference of a circle to its diameter (3.14159)
σ	Stefan-Boltzmann constant (5.673×10^{-8} Wm^{-2} K^{-4})
τ	transmittance to solar irradiation (dimensionless). *Subscript*: τ_d transmittance to diffuse skylight
ψ	zenith angle of the sun (degrees)
κ_a	thermal diffusivity of still air (2.22×10^{-5} m^2 s^{-1})
δ_{bl}	the thickness of the laminar boundary layer (m)
k	thermal conductivity coefficient (Wm^{-1} °C)

Roman Alphabet

$\bar{d}$	average distance between the Earth and the Sun (1.497×10^{11} m)
$\bar{S}_0$	solar constant (W m^{-2})

a'	calculated mean inclination angle (degrees)
A	constant, $(0.71)^{n}$ or total surface area of the plant part (m^{2}). *Subscripts*: $A_{T_{co}}$ total area of the corolla; $A_{H_{co}}$ horizontal projected area of the corolla
a	altitude angle of the sun (degrees). *Subscript*: a_s for the plant surface
a_i	absorptance of a surface to a particular source i of incident radiation (decimal fraction). *Subscripts*: a_l longwave; a_s shortwave
A_i	exposed area of the plant surface (m^{-2}). *Subscripts*: A_{l_s} to longwave from the sky; A_{l_g} to longwave from the ground; A_s to direct shortwave; A_r to reflected shortwave from the surroundings
ATP	adenosine triphosphate
Az	calculated mean azimuth,
B	constant
C	quantity of heat exchange by convection (W m^{-2})
c_p	specific heat capacity of air at constant pressure (1.012 kJ kg^{-1} °C^{-1}). *Subscript*: c_{p_t} of the plant tissue (3800 J kg^{-1} K^{-1})
d	characteristic dimension of the plant part (m)
D	Diameter of the corolla (m)
d	instantaneous distance between the Earth and the Sun (m)
E	flux of water vapour, evaporation rate or transpiration (kg m^{-2} s^{-1} or mg m^{-2} s^{-1}). *Subscripts*: E_G gravimetric
e	saturation vapour pressure (kPa). *Subscripts*: e_s at surface temperature; e_a of the surrounding air; e_d dry air; e_w wet air
Exp	exponential
g	acceleration due to gravity (9.81 m s^{-2}) or conductance (mmol m^{-2} s^{-1}). *Subscript*: g_a boundary layer conductance; g_s stomatal conductance
G	heat flow by conduction (W m^{-2})
h	hour angle (degrees). *Subscript*: h_c convective heat-transfer coefficient

	($W\ m^{-2}\ {}^{\circ}C^{-1}$)
$\boldsymbol{H}$	sensible heat loss ($W\ m^{-2}$)
Ho	null hypothesis whether accepted (a) or rejected (r)
i	angle of incidence (degrees) or bud interior
IR	infra-red
k	thermal conductivity coefficient of a material. *Subscript*: k_a of air at 30 °C ($2.64 \times 10^{-2}\ W\ m^{-2}\ K^{-1}$)
L	flux of longwave radiation ($W\ m^{-2}$). *Subscripts*: L_s thermal radiation from the sky; L_g thermal radiation from the ground
ln	logarithm to the base e (exp)
m	air mass (dimensionless) or constant
$\boldsymbol{M}$	metabolic heat storage ($W\ m^{-2}$)
n	constant or sample size
N	day of the year (1 - 365) or sample size ($N = N1 + N2$) where $N1$ and $N2$ are the sample size of the two populations being tested
o	ovary (female *Rhizanthes lowii*)
P	probability
p	atmospheric pressure ($N\ m^{-2}$). *Subscript*: p_0 atmospheric pressure at sea level
$\boldsymbol{R}$	radiation flux ($W\ m^{-2}$). *Subscripts*: $\boldsymbol{R}_n$ net radiation; $\boldsymbol{R}_a$ radiation absorbed; $\boldsymbol{R}_\varepsilon$ radiation emitted; $\boldsymbol{R}_i$ total radiation flux incident on a plant surface
$\boldsymbol{R}$	universal gas constant ($8.314\ J\ mol^{-1}\ K^{-1}$)
r	shortwave reflection coefficient (%), reflectance (decimal fraction). *Subscript*: r_g reflectance of the ground surface; r_a boundary layer resistance or aerodynamic resistance ($s\ m^{-1}$); r_s stomatal resistance ($s\ m^{-1}$).
RH	relative humidity of moist air (%)
s	bud surface
S	short wave radiation ($W\ m^{-2}$). *Subscripts*: S_p direct solar radiation; S_d

	diffuse radiation; S_g global radiation, S_s solar radiation incident on an inclined surface, S_r reflected radiation
T	a population where $T1$ and $T2$ are the two populations being tested or temperature (°C or K). *Subscripts*: T_a air $= T_d$ dry bulb; T_k apparent radiative temperature of the sky; T_g ground; T_s surface; T_w wet bulb; T_g greased; T_c control; T_{gy} gynoecia; T_{co} corolla; T_{se} sepal; T_{so} soil; T_{da} air inside the diaphragm; T_{pr} process; T_c centre of the column; T_{an} annulus, T_{at} anther (male); T_{st} stigmatic fascia (female); T_{us} upper surface
t	time (s or h)
u	wind speed (m s^{-1})
U^2	the test statistic, called the Watson U^2
UV	ultraviolet
Vis	visible light
X	rate of physical heat storage (W m^{-2})
Z	the test statistic, called Rayleigh's Z

Non-dimensional groups

Re	Reynolds number
Nu	Nusselt number
Pr	Prandtl number
Gr	Grashof number

Contents

Chapter 1

Introduction

The aim of this thesis is to explore some aspects of the functioning of flowers, having in mind that plants, like any other organisms are subject to physical principles and cannot violate physical laws. Within this biophysical framework, this study investigated the relationship between the flower's form and function, focusing on the thermal aspects of flowers in tropical environments. It shows that the flower's thermal relationships are not only important to the flower itself but also to the different associations between flowers and pollinators, and to the reproductive success of the plant. A unified approach is used to analyse the energy budgets of flowers belonging to different families *Rafflesiaceae* and *Convolvulaceae*, and discuss the broader ecological and evolutionary implications of their thermal regimes.

1.1 Context

Plants receive most of their thermal energy directly from the external environment. A plant exchanges energy with its environment through radiation, convection, conduction, and evaporation (Gates 1980). Its temperature is the net result of gains and losses of energy.

As the plant temperature increases, convection, conduction, re-radiation and usually evaporation also increase until the energy gains are balanced by energy losses, i.e. a steady state is reached and thermal equilibrium is achieved. The resulting tissue temperature may vary with position on the plant, according to the microclimate, and is determined by the *local* energy balance, acting on different organs. Extreme temperatures impact upon a suite of physiological processes (including photosynthesis and respiration) that lead ultimately to reproduction and may thus determine the limits of survival of the species (Watts 1977; Nobel 1978; Gates 1980; Grace 1989; Jones 1992; Singla, Pareek and Grover 1997).

Plants vary in their ability to withstand high and low temperatures, depending upon where they are growing. Plants adapted to grow in hot environments display mechanisms that prevent overheating and generally maintain temperatures close to the air temperature by decreasing net radiation absorbed through leaf movements and by increasing latent heat loss through transpiration. Some types of perennial

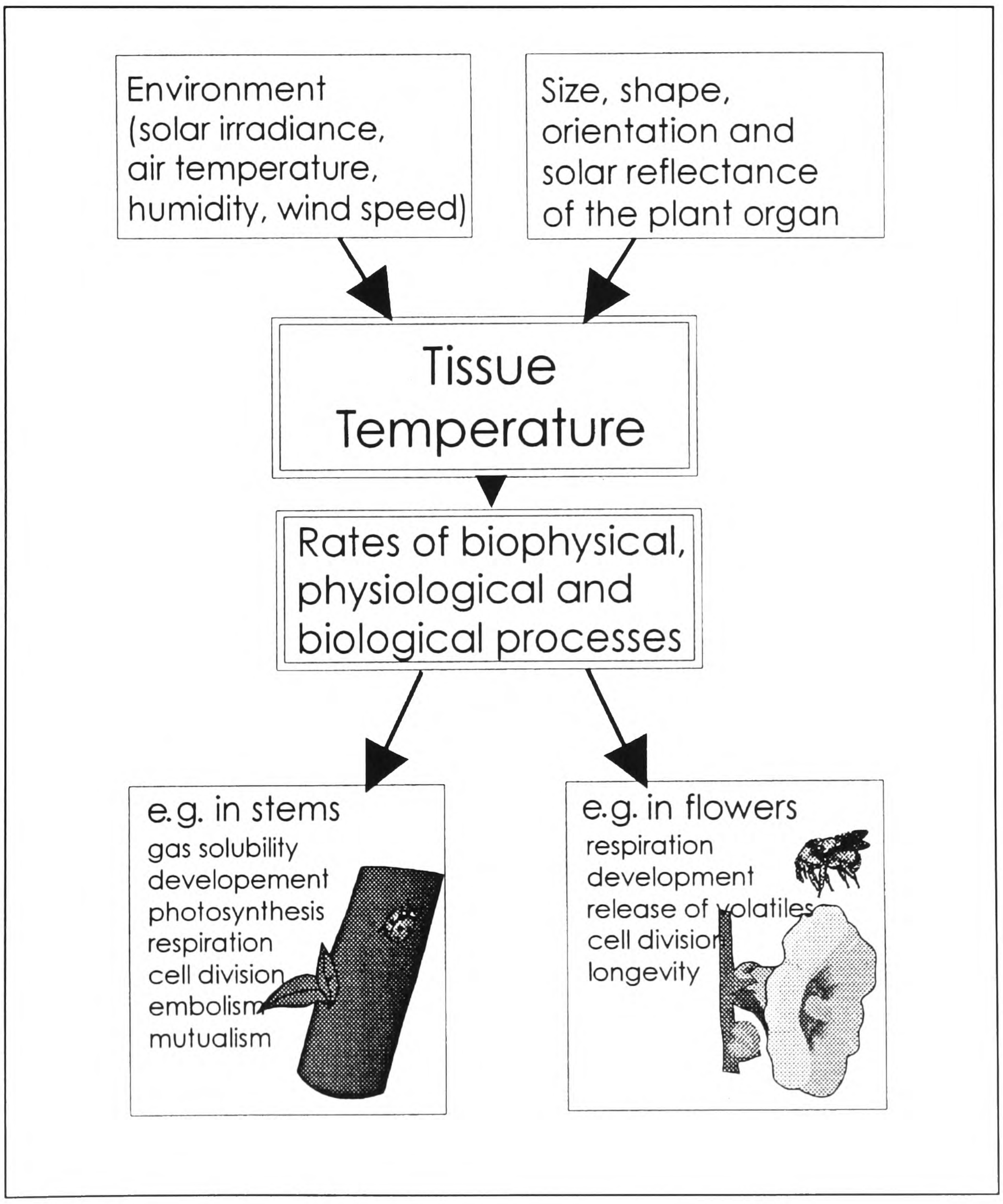

Figure 1. 1 A simple schematic representation of the interaction between the environment, and the form and function of plant organs i.e. stems and flowers.

species have drought-deciduous photosynthetic tissues, which avoid extreme temperatures, excessive irradiance levels, as well as water stress by abscission of those tissues (Ehleringer 1985). To tolerate high temperatures and the extreme conditions of drought that may accompany them, they may adjust their physiology, for example through the formation of heat shock proteins (HSPs) (Singla et al. 1997) and by tolerating cell dehydration (Kappen 1981).

In all cases plants produce heat by metabolic activity but only in rare cases is the heat produced substantial. Flowers and inflorescences belonging to the families *Cycadaceae*, *Nymphaeaceae*, *Aristolochiaceae*, *Araceae*, *Arecaceae*, *Cyclanthaceae*, *Anonaceae*, *Magnoliaceae*, and *Nelumbonaceae* are among the heat-producers.

1.2 Temperature control of plant organs

1.2.1 Leaves

There is a large literature on the environmental control of leaf temperature. Here, I mention the case of leaves rather briefly, as in this thesis the subjects of study are flowers. Of course, the principles are the same.

The temperature of a leaf is vital to its survival and determines the rate of

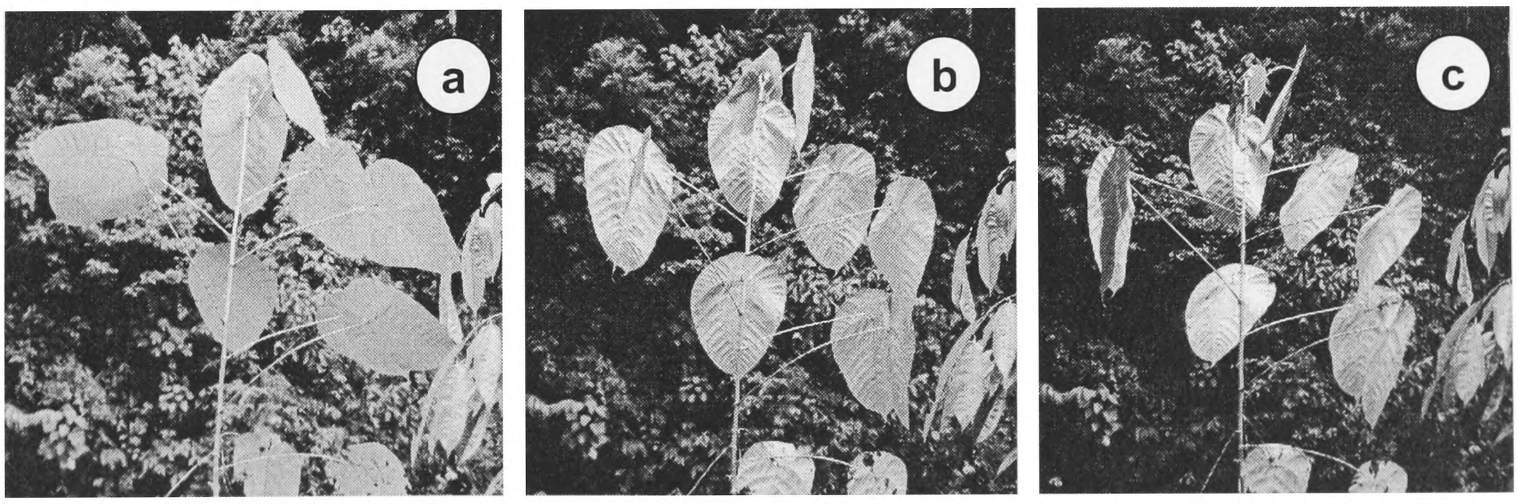

Figure 1. 2 *Macaranga lamellata* an ant-plant growing in a bank river in the rain forest in Borneo which adjusts the posture of the leaves possibly to avoid direct solar radiation (**a**) 9:48h, (**b**) 10:10h, (**c**) 10:50h.

biochemical and physiological processes. Photosynthesis is strongly affected by leaf temperature and high solar irradiation (Andrews and Muller 1985; Bao and Nilsen 1988). Many plants adjust the posture of their leaves and thereby avoid high insolation which may cause overheating or damage to photosynthesis (Adams and Strain 1968; Forseth and Ehleringer 1982; Ludlow and Björkman 1984; Werk and Ehleringer 1984; Prichard and Forseth 1988a; Donahue and Berg 1990; Berg and Heuchelin 1990; Fu and Ehleringer 1991; Rosa, Dillenberg and Forseth 1991; Kao and Forseth 1991; Kao and Forseth 1992; Alvino, Centritto and De Lorenzi 1994; Yu and Berg 1994; Rosa and Forseth 1996; Thanisawanyangkura, Sinoquet, Rivet et al. 1997). Particular studies on leaves showed that wind in combination with transpiration reduces leaf temperature (Drake, Raschke and Salisbury 1970; Grace, Fasehun and Dixon 1980; Naidoo and Von Willert 1994; Cho and Kwack 1996).

There have been a number of studies on the energy balance and temperature regulation in leaves and there exist algorithms for estimating the leaf temperature in a defined environment (Raschke 1956; Gates 1962; Gates 1965; Gates and Papian 1971; Miller 1971; Campbell 1977; Gates 1980; Chiariello 1984; Althawadi and Grace 1986; Leuning 1989; Monteith and Unsworth 1990; Nobel 1991; Van Gardingen and Grace 1991).

Studies on the morphology of the leaves suggest that pubescence (Meinzer, Goldstein and Rundel 1985; Meinzer and Goldstein 1985) size and shape (Parkhurst and Loucks 1972; Taylor 1975), and high reflectance (Levitt 1980; Ehleringer 1981) are all involved in maintaining leaves close to their physiologically optimal temperatures.

Additional studies show how leaf properties such as inclination, orientation (Forseth and Teramura 1986), wilting (Chiariello, Field and Mooney 1987), leaf rolling, and leaf movements (Ehleringer and Forseth 1980; Bao and Nilsen 1988;

Prichard and Forseth 1988b) influence the heat load and may reduce the leaf temperature in hot environments.

Other organs, such as flowers and stems are subject to the same principles but very little attention has been devoted to them, especially in the tropical environment.

1.2.2 Flowers

Angiosperms produce flowers in order to reproduce. The majority of species attract pollinators by visual or olfactory signals; at the same time, pollinators benefit from the flowers by obtaining food and digestive components, and promoting their own reproductive behaviour. Consequently, when plants produce flowers with attractive and exposed petals, these structures must be maintained within physiologically acceptable physical conditions.

Flowers that need to be warm

In the alpine, boreal and temperate zones there are many flowers which open when the air temperature is low. Selection has acted on these flowers in various ways. Some posses the appropriate shape to capture solar radiation, with the corolla acting as a parabolic antenna (Kevan 1975; Knutson 1981), other flowers track the sun (Hocking 1968; Kjellberg, Karlsson and Kerstensson 1982; Kudo 1995; Totland 1996; Krannitz 1996), while others grow in a convenient position in the plant (Lu, Rieger and Duemmel 1992) or posses pubescent floral structures (Miller 1986) or have suitable colours that help them to increase the gynoecium temperature (Molgaard 1989). This increase in temperature may promote growth and development of the reproductive parts and when pollinated, contribute to pollen germination and enlargement of the pollen tube (Kevan 1975). Most of these flowers are entomophilous i.e they are pollinated by insects and this gain in heat, at the same time, may serve as an energy reward for the pollinators (Hocking and Sharplin 1965; Hocking 1968).

One of the most common ways that entomophilous flowers use to signal their presence and attract pollinators is the production of specific odours (Proctor and Yeo 1973; Proctor, Yeo and Lack 1996). In a diversity of pollination syndromes, for example pollination by flies (Meeuse 1966), beetles (Thien, Heimermann and Holman 1975; Armstrong 1997), fig wasps (Barker 1985), bats (Knudsen and Tollsten 1995), and bees (Dobson, Dressler, Adams et al. 1969) pollinator attraction to flowers is due to the emission of a complex mixture of aromatic compounds by the flower (Faegri and van der Pijl 1979; Pellmyr and Thien 1986). Studies on *Ribes nigrum* (Hansted, Jakobsen and Olsen 1994) and *Trifolium repens* (Jakobsen and Olsen 1994), showed that temperature has a strong effect on the qualitative composition of the volatiles emitted. This suggests that only a specific (qualitative and quantitative) combination of the compounds may ensure the attraction of pollinators and that flowers need an optimum temperature to release the right combination of volatiles that attract their pollinators.

Flowers of some species (*Sauromatum guttatum*, *Arum italicum, A. maculatum*, *Monstera deliciosa*, *Victoria cruziana*, *V. amazonica*, *Nelumbo lutea*, among others) produce their own heat by increasing metabolic activity (Lamark 1778; Meeuse 1975; Helm, Petersen and Abernethy 1989; Skubatz, Tang and Meeuse 1993). To date, it has been claimed that four of these thermogenic flowers (*Philodendron selloum*, *Simplocarpus foetidus*, *Xantosoma robustum* and *Nelumbo nucifera*) actually regulate their temperature. Their internal temperature is kept nearly constant in spite of large fluctuations in air temperature (Nagy, Odell and Seymour 1972; Knutson 1974; Meeuse and Raskin 1988; Uemura, Ohkawara and Kudo 1993; Seymour and Schultze-Motel 1996). Production of heat and thermoregulation seem to be associated with growth, survival and reproduction in these species.

Some species of the parasitic family *Rafflesiaceae* are adapted to live in the understory of the tropical rain forest where the ambient conditions are nearly

Figure 1. 3 *Rafflesiaceae* species growing in the understory of the rain forest in Borneo. *Rafflesia tuan –mudae* growing in Sarawak, Malaysia (left); *Rhizanthes lowii* growing in Brunei (right).

constant (high relative humidity, low incident radiation and relatively constant air and soil temperature). These flowers release volatile compounds to attract carrion flies that effect pollination, and is said that they resemble rotting flesh. It may be that these flowers have the capability of generating heat, as part of their mimicry. As they are parasitic, they have the advantage over most other species as the respiratory substrate is derived from the host plant. Part of this study will focus on flowers in the *Rafflesiaceae*.

Flowers that need to be cool

In contrast to the cases mentioned above, species occupying open habitats may experience very high solar radiation combined with high air temperature. This occurs in tropical and arid environments. However there are not many examples in the literature that illustrate how tropical flowers respond to these high temperatures. Studies on several species of cultivated plants, including pea (Guilioni, Wery and Tardieu 1997), tomato (Levy, Rabinowitch and Kedar 1978), beans (Monterroso and Wien 1990; Konsens, Ofir and Kigel 1991), cowpea

(Ahmed, Hall and DeMason 1992; Ahmed and Hall 1993), and cotton , show that a short period of heat stress causes a significant increase in abortion frequency of floral buds and flowers. High temperature has been also shown to affect floral development and fruit set (Beppu, Okamoto, Sugiyama et al. 1997), delay flowering and produce abnormal flowers (Cockshull and Kofranek 1994; Matsui, Omasa and Horie 2000). It has been suggested that high temperature and humidity within the floral chamber may have a negative effect on pea growth (Nonnecke, Adedipe and Omrod 1971) and could also affect pollinators in naturally growing flowers (Cruden, Kinsman, Stockhouse II et al. 1976; Martinez del Rio and Burquez 1986). Studies of the Avocado inflorescence *(Persea americana)* revealed that there is an increase in transpiration associated with flowering, making flowering particularly expensive in the use of water. Although the functional significance of this extra transpiration was not discussed (Whiley, Chapman and Saranah 1988; Blanke and Lovatt 1993), it may relate to cooling. The importance of transpiration in temperature regulation is clearly demonstrated in the *Ficus* inflorescence (figs). Figs have to regulate temperature by transpiration to maintain suitable low internal temperatures for the development of the pollinator wasps.

Figure 1.4 Merremia borneensis (left) and Ipomoea pes-caprae (right), tropical convolvulaceous plants growing near the ground with flowers exposed to high solar radiation

Temperature increases of a just few degrees above ambient are enough to induce mass mortality in the wasps (Patiño, Herre and Tyree 1994).

It is not clear whether flowers in the tropics or other hot environments present adaptations to prevent overheating. They may for instance control temperature by evaporative cooling, at the expense of using water.

Many tropical plants are adapted to live in open areas and produce flowers near the ground, which are exposed to high solar radiation and extremely high air temperatures (Figure 1.4), Do these flowers have a cooling mechanism to protect the reproductive parts, and if so, What is the nature of this mechanism?

1.3 Objectives

In this thesis I focus on the thermal relationships of two specific flower types, where it seemed possible to find a connection between temperature of flowers and the behaviour of pollinators.

I aim to test the hypothesis that parasitic flowers of the *Rafflesiaceae*, that grow in the understory of the rain forest, respond to the low net radiation by producing their own heat. I also aim to investigate how some tropical flowers of the Convolvulaceae maintain their tissue temperatures at physiologically acceptable levels despite the high solar radiation and air temperature in their natural environment. The central questions which I address in this thesis are as follows.

(1) The flowers of the parasitic *Rafflesiaceae* occur in the dense shade of the forest understory. They attract pollinators by resembling rotting flesh and producing volatile substances, a form of mimicry. Does the mimicry include the production of heat?

(2) The flowers of the Convolvulaceae are generally numerous, short lived, and produced in bright sunshine. They attract pollinators by being visually conspicuous

but to achieve this they must face the sun. Do these flowers have a cooling mechanism, and if so, what is the nature of the mechanism?

(3) To what extent can flower temperature be said to be controlled?

I hope to use a unified approach to analyse the energy budgets of the study flowers, and I want to explore the broader ecological and evolutionary implications of their thermal regimes.

1.4 Outline of thesis

This thesis is composed of seven chapters. Following this introductory chapter (Chapter 1), the energy balance theory is presented (Chapter 2) and the physical terms and relationships used in each subsequent case study are defined. The third chapter describes the endothermic and thermoregulatory patterns of *Rhizanthes lowii* and discusses the possible implications on the pollination syndrome. The fourth chapter shows that *Rafflesia tuan-mudae* is an endothermic flower, and a detailed analysis of the heat production is presented. The implications of this endothermic pattern in terms of the pollinator attraction are discussed. The fifth chapter investigates the thermal regime of convolvulaceous flowers that are exposed to high solar radiation and shows how they respond to high solar irradiance. The sixth chapter provides evidence of heliotropism in tropical convolvulaceous flowers, the relevance of this pattern in terms of the flower's physical properties and the relationship with the pollinators are discussed. Chapter 7 contains the general discussion of this thesis.

Chapter 2

Theory

To explore the thermal relationships of flowers or any other plant organ it is necessary to refer to the principle of the conservation of energy (First Law of Thermodynamics) which states that energy cannot be created or destroyed but only changed from one form to another. Energy input to the plant organ, mainly solar radiation, is converted to heat, used in evaporation and used to create chemical bonds in photosynthesis. Energy influxes are balanced against effluxes by changes in plant temperature (Gates 1962; Gates 1980). This is best described by the energy budget of the plant.

Energy budget

The temperature of a plant surface is determined by the local energy budget, which adjusts under given environmental conditions until the heat gained is equal to the heat lost. The energy budget may be expressed as follows (Campbell 1977; Gates 1980; Grace 1983; Jones 1992; Monteith and Unsworth 1990; Nobel 1991):

$$\boldsymbol{R}_{\mathrm{n}} + \boldsymbol{M} + \boldsymbol{H} + \lambda\boldsymbol{E} + \boldsymbol{X} = 0 \tag{2.1}$$

Each term in the equation may be positive or negative depending on whether the exchange is *into* the surface or *out of* the surface. $\boldsymbol{R}_{\mathrm{n}}$ is the heat gained from radiation known as the net radiation, $\boldsymbol{M}$ (metabolism) is the net energy exchanged when solar energy is converted into chemical bonds in photosynthesis, or the heat released by respiration when energy-rich compounds are broken down, $\boldsymbol{H}$ is the convective and conductive heat loss (often called sensible heat), $\lambda\boldsymbol{E}$ is the net latent heat loss by evaporation (being the latent heat of vaporisation, λ multiplied by the evaporation rate E) and X is the net energy put into or taken out of physical storage within the plant. In this thesis I will express these fluxes per unit area of plant surface (usually projected onto a plane) to give units of flux density (W m^{-2}).

2.1 Net Radiation (R_n)

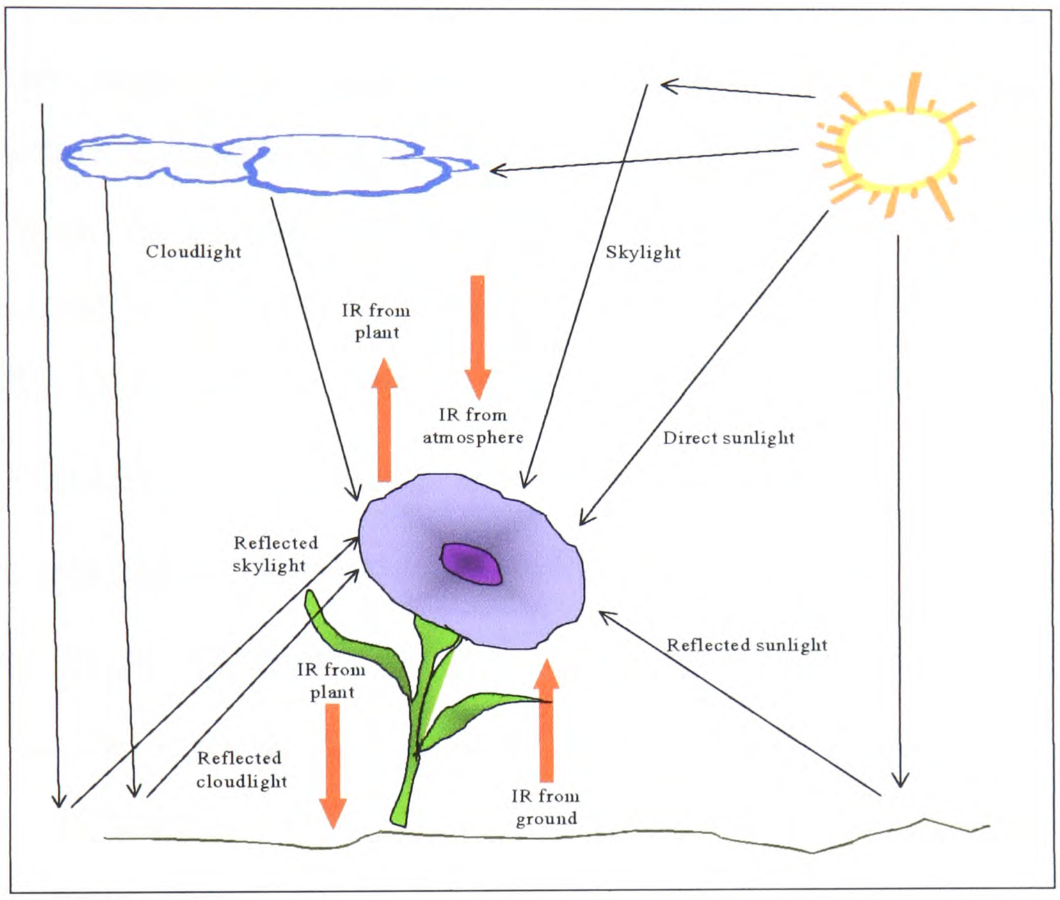

Figure 2. 1
Illustration of the fluxes of radiant energy to and from a flower, including shortwave radiation and infrared radiation.

The heat gained from radiation can be expressed as:

$$R_n = R_a - R_e \tag{2.2}$$

where R_a is the radiation absorbed including absorbed solar irradiation (shortwave radiation W m^{-2}, in the wave band 1.0 x 10^{-3} - 7.4 x 10^{-4}µm)[*] and absorbed infrared irradiation from the surroundings (longwave radiation W m^{-2}, in the wave band 7.4 µm)* and R_ε is the emitted infrared radiation.

Radiation absorbed R_a .

The radiation absorbed by a plant part, such as a flower, is estimated from the total flux of shortwave radiation (direct sunlight plus the diffuse radiation, and reflected radiation from the ground) and longwave radiation from the atmosphere. These energy streams are incident on both the upper and the lower surface of the plant and are affected by latitude, time of year and time of day (Fig. 2.1).

The total irradiation comprising the direct solar irradiation, S_p, plus diffuse shortwave irradiation (cloudlight and skylight), S_d, incident on a horizontal surface is referred to as the global irradiation, S_g (Fig 2.1). The global irradiation depends on the time of day, time of year, latitude, altitude and atmospheric conditions (Campbell 1977; Nobel 1991). Geometrical considerations are important: many plants adjust the angles of leaves and flowers and may exert coarse control of the temperature of their surfaces in this way (Berg and Heuchelin 1990; Ehleringer and Forseth 1980).

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[*] The energy for life originates in the form of electromagnetic radiation from the sun. Sunlight includes the entire range of radiation known as the electromagnetic spectrum. The electromagnetic spectrum is a broad band of radiant energy which extends over a range of wavelengths including gamma rays
(10^{-5} – 10^{-4} µm), X-rays (10^{-4} – 10^{-2} µm), ultraviolet radiation (10^{-2} – 4 x 10^{-} m), visible light (0.4 – 0.7 µm), infrared radiation (0.7 – 1000 µm), microwaves (1000 – 10 x10^{4} µm), etc. Some of this radiant energy is incident on the earth and only a small fraction is absorbed by plants.

For the energy balance of a plant part, exposed to solar radiation, the wavelengths include some of the ultraviolet (UV, 10 x 10^{-4} – 4 x 10^{-2} µm), the visible spectrum (Vis, 4 x 10^{-2} – 7.4 x 10^{-2} µm), and the infra-red (IR, 7. 4 x 10^{-2} – 10 µm)

Although not all the following equations (2.3 - 2.12) were used in this thesis, they are kept to maintain a logical sequence in the derivation of the energy balance of a plant part. Nevertheless, these equations will led to the derivation of the cos *i* (equation 2.13) using the geometrical relationship between the earth and the sun (Fig. 2.2, Box 2.1). The cos *i* will be applied in Chapter 6.

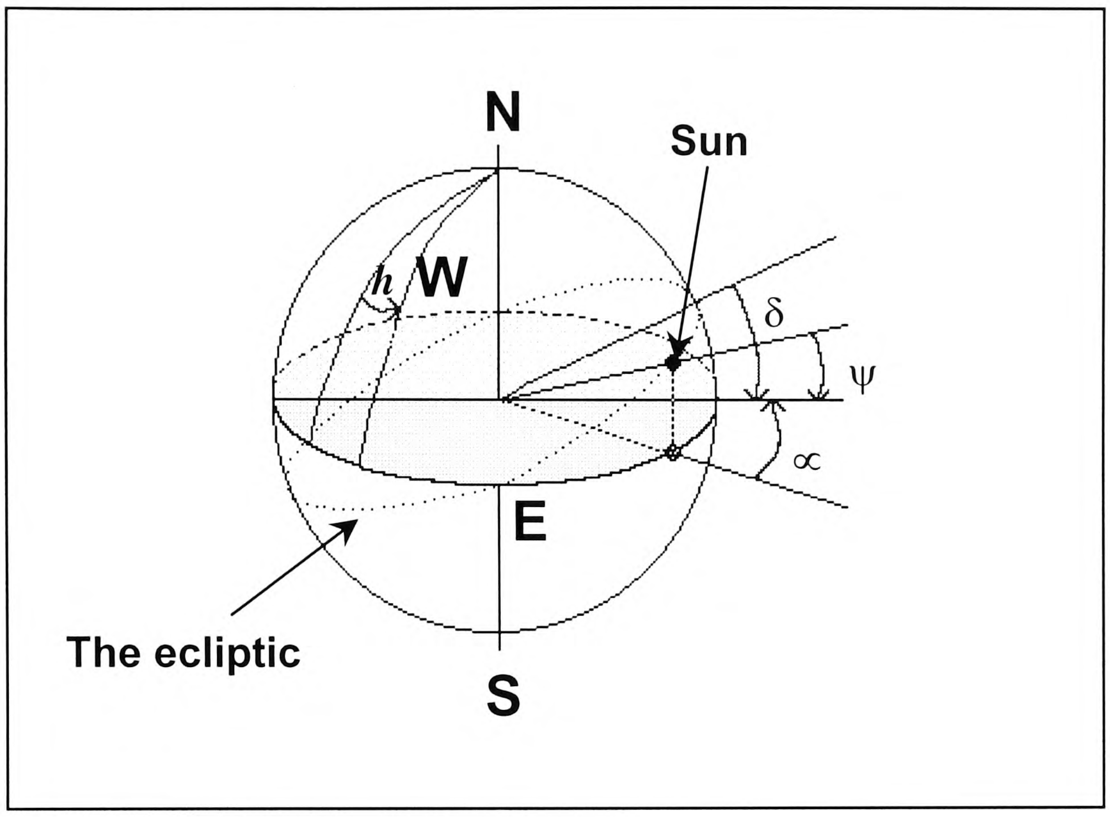

Figure 2. 2
Geometrical relationships of the solar position: azimuth ∝ , solar declination δ , hour angle *h* , and ψ zenith angle.

Direct Solar Radiation S_p

The instantaneous flux of direct solar irradiation (through a sky without clouds) incident upon a plant surface perpendicular to the beam can be written as follows (Gates 1980):

$$S_p = \bar{S}_0 \left(\frac{\bar{d}}{d} \right)^2 (\sin\varphi \sin\delta + \cos\varphi \cos\delta \cos h)\tau^{m} \qquad (2.3)$$

Where $\overline{S}_0$ is the solar constant[1], d is the distance of the earth from the sun which varies with the time of year, $\overline{d}$ is the mean value of the distance of the earth from the sun (1.497 x 10^{11} m) (Gates 1980 pp 99), φ is the latitude in degrees, δ is the solar declination in degrees i.e. the angle between the equatorial plane and the ecliptic (Fig. 2.2, Box 2.1a), h is the hour angle, describing the difference between local solar time and solar noon (Box 2.1b), τ is the transmittance to solar radiation of the atmosphere (the fraction of sunlight transmitted when the sun is at the zenith). Values of atmospheric transmittance range from about 0.4 (depending of the turbidity) at sea level to 0.8 at high elevations with an extremely clear dry sky (Lucas and Foster 1999), and m is known as the air mass and is a measure of the path length through which the solar beam passes (Box 2.1c).

$$S_{\mathrm{d}} = \tau_{\mathrm{d}} S_{\mathrm{p}} \tag{2.10}$$

The total short-wave radiation (also called global radiation, S_{g}) incident on a horizontal plant part is the diffuse shortwave radiation plus the direct solar radiation and may be expressed as follows

$$S_{\mathrm{g}} = S_{\mathrm{p}} + S_{\mathrm{d}} = S_{\mathrm{o}}\tau^{\mathrm{m}} \cos\psi + S_{\mathrm{o}}(0.271 - 0.294\tau^{\mathrm{m}})\cos\psi \tag{2.11}$$

For surfaces with inclinations other than horizontal and with different azimuths (for example the corolla of a flower), the total short-wave radiation becomes

$$S_{\mathrm{s}} = S_{\mathrm{o}}\tau^{\mathrm{m}} \cos i \tag{2.12}$$

where i is the angle of incidence between the direct beam of sunlight and the normal to the surface. The cosine of the angle of incidence (cos i) represents the

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[1] The solar constant, $\overline{S}_0$ is defined as the irradiance of a surface area held perpendicular to the solar beam at the mean distance of the earth from the sun (1.50 x 10^8 m). The solar constant ranges between 1369 and 1375 W m^{-2} with a mean value of 1373 W m^{-2} (Monteith and Unsworth 1990 pp 36). It is the solar irradiation incident on the earth's atmosphere.

cosine of the angle between a normal to the sun's direct beam and the corolla of the flower.

Box 2. 1 Definitions of the terms of the equation 2.3.

a. The declination angle, δ, is described for the northern hemisphere as:

$$\delta = 23.45\cos\left(\frac{2\pi\,(N-172)}{365}\right) \qquad (2.4)$$

where N is the day of the year.

b. The hour angle, h, is defined as:

$$h = (t-12)\left(\frac{\pi}{12}\right) \qquad (2.5)$$

where t is the mean solar time in hours (Ehleringer 1989 pp 117).

c. The air mass m is defined as follows:

$$m = \frac{\left(\frac{p}{p_0}\right)}{\cos\psi} = \frac{\left(\frac{p}{p_0}\right)}{\sin a} \qquad (2.6)$$

where the ratio $\frac{p}{p_0}$ is atmospheric pressure at the irradiated point divided by the atmospheric pressure at sea level, and corrects for altitude effects, ψ is the zenith angle of the sun, and a is the altitude of the sun (the elevation of the sun above the horizon at the irradiated point). The altitude of the sun, a, is dependent upon latitude of the irradiated point (φ), declination of the sun (δ), hour angle of the sun (h), and azimuth ($\propto$) of the sun (representing the compass direction). Spherical trigonometry is used to show the relationship between these variables.

$$\cos\psi = \sin\varphi\,\sin\delta = \cos\varphi\,\cos\delta\,\cos h \qquad (2.7)$$

$$\sin a = \sin\varphi\,\sin\delta + \cos\varphi\,\cos\delta\,\cos h \qquad (2.8)$$

$$\sin\propto = \frac{-\cos\delta\,\sin h}{\cos a} \qquad (2.9)$$

It is a measure of the proportion of the direct beam incident on the corolla. A cos i of 1 would represent a corolla with the adaxial surface perpendicular to the direct beam, while a value of 0 would represent a corolla with its adaxial surface parallel to the direct solar beam.

$$\cos i = \cos a_s \cos a + \cos(\alpha - \beta) \sin a_s \sin a \tag{2.13}$$

where a_s is the altitude angle for the plant surface and β is the azimuth angle of the plant surface.

Reflected Radiation (S_r)

The reflected sunlight, cloudlight and skylight by the surroundings often amounts 10% to 30% of the global irradiation. For the whole solar spectrum, the fraction of the incident shortwave irradiation reflected, r (reflection coefficient) from a natural surface is often termed *albedo*. Values of shortwave reflection coefficients for different natural surfaces are found in several texts (Monteith and Unsworth 1990). The reflected radiation is defined as follows:

$$S_r = rS_s \tag{2.14}$$

where S_s is the total short-wave radiation.

Longwave radiation L

A plant receives thermal radiation from the sky above and from the ground below. According to (Swinbank 1963) the thermal radiation coming down from clear sky (L_s) can be approximated as (Gates 1980 pp 152):

$$L_s = 53.1 \times 10^{-14} (T_a + 273)^6 \tag{2.15}$$

The thermal radiation emitted by the ground (L_g) can be calculated from the blackbody radiation law, then, the total thermal radiation gives

$$L = L_\mathrm{s} + L_\mathrm{g} = \sigma\left[\left(T_\mathrm{k} + 273\right)^4 - \left(T_\mathrm{g} + 273\right)^4\right] \tag{2.16}$$

where σ is the Stephan-Boltzmann constant (5.673 x 10^{-8} W m^{-2} K^{-4}), T_k (°C) is the apparent temperature of the sky and T_g (°C) is the temperature of the ground. The temperature of the ground can be measured directly with thermometers and thermocouples, and the temperature of the sky can be estimated from the following equation (Gates 1980 pp 154):

$$T_\mathrm{k} = T_\mathrm{a} - 20.4 + 0.22T_\mathrm{a} \tag{2.17}$$

where T_a is the air temperature in °C.

Adding the total longwave and shortwave radiation gives the total radiation flux incident on a plant surface

$$R_\mathrm{i} = L_\mathrm{s} + r_\mathrm{g}L_\mathrm{g} + S_\mathrm{s} + rS_\mathrm{s} \tag{2.18}$$

where r_g is the reflectance of the ground surface.

A plant part absorbs a fraction of the total radiation flux incident on it, depending on shape, orientation and absorptance. The total amount of radiation absorbed by a plant depends on the surface area exposed A_i to each source of radiation, and on its respective absorptance, a_i.

$$R_\mathrm{a} = \frac{\left[a_\mathrm{l}\left(A_\mathrm{ls}L_\mathrm{s} + A_\mathrm{lg}L_\mathrm{g}\right) + a_\mathrm{s}\left(A_\mathrm{s}S_\mathrm{s} + rA_\mathrm{r}S_\mathrm{s}\right)\right]}{A} \tag{2.19}$$

where A is the total surface area of the plant part (Gates 1980 pp 166).

Radiation emitted, R_ε

The emitted infrared radiation R_ε (per unit area) by the surface of the plant part is proportional to the fourth power of the absolute temperature of the surface T_s and according to the emissivity ε of the surface. For the flowers, ε was assumed to be 0.96 (Nobel 1991):

$$R_\varepsilon = \varepsilon\sigma\,[T_s]^4 \tag{2.20}$$

2.2 Sensible Heat Loss (H)

The sensible heat loss, $\boldsymbol{H}$, is the sum of all heat loss to the surroundings by conduction or convection:

$$\boldsymbol{H} = \boldsymbol{G} + \boldsymbol{C} \tag{2.21}$$

where $\boldsymbol{G}$ is the rate of heat exchanged by conduction from and to other surroundings (such as the soil and is usually small) to the plant part and $\boldsymbol{C}$ is the convective heat flux which is the transfer of heat by moving air.

Heat convection (C)

Heat transfer by convection occurs by two distinct processes: forced convection or transfer of heat through the boundary layer of the surface exposed to an airstream, proceeding at a rate which depends on the velocity of the flow; and free convection which depends on the ascent of warm air above heated surfaces or the descent of cold air beneath cooled surfaces, both a result of differences in air density (Monteith and Unsworth 1990). When the surface of the plant part is experiencing laminar flow (low Re, defined below) the heat transfer per unit area ($\boldsymbol{C}$) can be written as:

$$C=\frac{k_a}{\delta_{bl}\left(T_s-T_a\right)} \tag{2.22}$$

where k_a is the thermal conductivity of air (0.026 W m^{-1} K^{-1}), δ_{bl} is the thickness of the laminar boundary layer, and T_s is the temperature of the plant part, T_a is the temperature of the air where:

$$\frac{k_a}{\delta_{bl}}=h_c=\frac{\rho c_p \kappa_a}{\delta_{bl}} \tag{2.23}$$

The convective-heat transfer coefficient (h_c) of the surface of the plant part is a complex function of the size, shape, orientation of the plant, being influenced by the fluid density (ρ), viscosity (μ), specific heat at constant pressure (c_p), and thermal diffusivity of the air (κ_a) and wind speed (u). An analogous expression would work for turbulent flow (high Re), with the important difference that the heat is transported by turbulent diffusion, and so κ_a would underestimate the transfer rate. The functional relationship among these variables is simplified by using non-dimensional groups of quantities and a description of these groups is necessary for a comparison of the convective heat loss from plant parts of similar shapes or geometry but different sizes and for different wind speeds.

Non-dimensional groups

Non-dimensional groups were used in the analysis of convective heat loss of the flowers *Rhizanthes lowii* (Chapter 3) and *Rafflesia tuan-mudae* (Chapter 4).

The Reynolds number (Re) expresses the dynamic properties of the air indicating whether the flow over the plant part surface is laminar or turbulent in character. In general when the Re number is small, flow is laminar; when it is large, flow is turbulent (Gates 1980). The transition from laminar to turbulent flow is said to occur at values as low as 8×10^4 or as high as 10^6, but the critical value of the Re is influenced by roughness and topography of the surface (Grace and Wilson 1976). Re is defined as follows (Gates 1980 pp 280):

$$\mathrm{Re} = \frac{ud}{\nu} = \frac{ud\rho}{\mu} \tag{2.24}$$

where u is the wind speed, d is the dimension or length of the surface over which air has passed, ν is the kinematic viscosity coefficient of air ($m^2\ s^{-1}$), ρ is the density of air ($kg\ m^{-3}$) and, μ is the coefficient of viscosity or dynamic viscosity ($N\ s\ m^{-2}$). Some typical values of Re for the understory flowers of *Rhizanthes lowii* and *Rafflesia tuan-mudae* are presented in Table 2.1. The values of Re indicate that laminar flow is probable for these structures.

Table 2.1 Values of non-dimensional numbers for *Rhizanthes lowii* and *Rafflesia tuan-mudae*, based on measured wind speeds in this study.

Species	**u ($m\ s^{-1}$)**	**Re**	**Nu_{free}**	**Nu_{force}**	**Gr**
Rhizanthes	0.13 – 0.47	933	0 – 110.3	2.6	2.4×10^7
Rafflesia	0 – 1.18	$0 - 3.4\times10^4$	8.6 – 16.3	20.6	$-2.1\times10^{-7} - 7.8\times10^{-7}$

The Nusselt number (Nu) is a useful way of comparing the forces associated with geometrically similar bodies immersed in a moving fluid, it provides a basis for comparing rates of convective heat loss from similar bodies of different size exposed to different wind speeds (Monteith and Unsworth 1990 pp 122):

$$\mathrm{Nu} = \frac{d}{\delta_{bl}} \tag{2.25}$$

where d is the characteristic dimension and δ_{bl} is the thickness of the boundary layer. Expressions for the thickness of the boundary layer are summarised in Table 2.2.

For a particular plant part with a characteristic dimension d the heat transfer across the boundary layer is (Dixon and Grace 1983):

$$C = \frac{\left(\frac{d}{\delta_{\mathrm{bl}}}\right)\rho c_{\mathrm{p}}\kappa_{\mathrm{a}}\left(T_{\mathrm{s}} - T_{\mathrm{a}}\right)}{d} \tag{2.26}$$

$$\mathrm{Nu} = \frac{Cd}{\rho c_{\mathrm{p}}\kappa_{\mathrm{a}}\left(T_{\mathrm{s}} - T_{\mathrm{a}}\right)} \tag{2.27}$$

Some values of Nu for *Rhizanthes lowii* and *Rafflesia tuan-mudae* are presented in Table 2.1.

The rate of convective heat transfer in air can be written as

$$C = \frac{\rho c_{\mathrm{p}}\left(T_{\mathrm{s}} - T_{\mathrm{a}}\right)}{r_{\mathrm{a}}} \tag{2.28}$$

where r_{a} (s m^{-1}) is the boundary layer or aerodynamic resistance for the convective heat transfer. **Nu** can be related to r_{a} from the equations (2.26) and (2.28), it gives (Monteith and Unsworth 1990 pp122):

$$r_{\mathrm{a}} = \frac{d}{\kappa_{\mathrm{a}}\mathrm{Nu}} = \frac{d\rho c_{\mathrm{p}}}{\kappa_{\mathrm{a}}\mathrm{Nu}} \tag{2.29}$$

The aerodynamic resistance, r_{a} can be calculated as a hybrid of the resistance for force and free convection applying the parallel resistance analogue (Grace 1983):

$$r_{\mathrm{a}} = \frac{1}{\left(\frac{1}{r_{\mathrm{a\,(forced)}}} + \frac{1}{r_{\mathrm{a\,(free)}}}\right)} \tag{2.30}$$

where

$$r_{\mathrm{a(forced)}} = \frac{d}{\kappa_{\mathrm{a}} \mathrm{Nu}_{\mathrm{(forced)}}} \tag{2.31}$$

$$r_{\mathrm{a\,(free)}} = \frac{d}{\kappa_{\mathrm{a}} \mathrm{Nu}_{\mathrm{(free)}}} \tag{2.32}$$

In free convection heat transfer depends on the circulation of air over and around the plant part, maintained by gradients of temperature which create gradients of density. In this case Nu is a function of the Grashof number (Gr) and the Prandtl number (Pr) (Monteith and Unsworth 1990 pp 123). Gr is used when free convection dominates forced convection, i.e. at very low wind speed and large differences between air and surface temperature. Gr indicates the ability of a parcel of air warmer or colder than the surrounding air to rise against or fall with the attractive force of gravity (Dixon and Grace 1983; Gates 1980 pp 281):

$$\mathrm{Gr} = \frac{\alpha \rho^2 g d^3 \left(T_{\mathrm{s}} - T_{\mathrm{a}}\right)}{\mu^2} = \frac{\alpha g d^3 \left(T_{\mathrm{s}} - T_{\mathrm{a}}\right)}{\nu^2} \tag{2.33}$$

where α is the coefficient of thermal expansion of air, g is the acceleration due to gravity, d represents the characteristic dimension of the plant part.

The Prandtl number (Pr) expresses the static properties of the air. It is defined by (Gates 1980 pp 281):

$$\mathrm{Pr} = \frac{c_{\mathrm{p}} \mu}{\kappa_{\mathrm{a}}} = \frac{\nu}{\kappa_{\mathrm{a}}} \tag{2.34}$$

Physically, Pr represents the ratio between the molecular diffusivities of momentum and heat (Gates 1980 pp 281). For air Pr = 0.71 (Monteith and Unsworth 1990 pp 123).

Then Nu is equivalent to (Monteith and Unsworth 1990 pp 123):

$$\mathrm{Nu} = B\mathrm{Gr}^{\mathrm{m}} \tag{2.35}$$

where $B = \mathrm{Pr}^{\mathrm{m}} = (0.71)^{\mathrm{m}}$. Values of the constants B and m which depend on geometry are given in Monteith and Unsworth (1990, Table A5 (b) pp 271).

In forced convection, Nu depends on the rate of heat transfer across the air boundary layer from a plant part surface hotter or cooler than the air passing over it (Monteith and Unsworth 1990 pp 122).

$$\mathrm{Nu} = A\mathrm{Re}^{n} \tag{2.36}$$

where $A = \mathrm{Pr}^{\mathrm{n}} = (0.71)^{\mathrm{n}}$. Values of the numerical constants A and n for different types of geometry are given in Monteith and Unsworth (1990, Table A 5(a) pp 269).

Table 2.3 gives the relationships of Nu for air used in this thesis for different types of geometry depending on the flower part (i.e. bud and gynoecium= sphere, corolla= flat plate, column= cylinder).

Nu can also be expressed in terms of the familiar boundary layer conductance g_a, much used by plant physiologists (Van Gardingen and Grace 1991):

$$\mathrm{Nu} = \frac{g_{\mathrm{a}} d R T_{\mathrm{a}}}{p \kappa_{\mathrm{a}}} \tag{2.37}$$

where, d represents the characteristic dimension of the object parallel to the flow, p is the atmospheric pressure (N m^{-2}), and R is the universal gas constant (8.314 J mol^{-1} K^{-1}).

Table 2.2 Equations to calculate the thickness of the boundary layer (m) according with the shape of different parts of the flowers.

Geometry	Condition	Equation	
Plane surface e.g.. corolla	laminar flow, Re up to 10^4 (Gates 1980 pp 286)	$\delta_{bl} = 5d\,\mathrm{Re}^{-0.5}$	(2.38)
Plane surface e.g.. corolla	turbulent flow, Re between 10^4 and 10^7 (Gates 1980 pp 289)	$\delta_{bl} = 0.376d\,\mathrm{Re}^{-0.2}$	(2.39)
Spherical surface (such as flower bud)	wind velocity measured near the surface (Patiño et al 1994) adapted from (Nobel 1975)	$\delta_{bl} = 1.1\times10^{-3}\left(2.8\left[\frac{d}{u}\right]^{0.5} + \frac{0.25}{u}\right)$	(2.40)

2.3 Latent heat loss (λE)

The rate at which water evaporates from a "wet" plant surface is defined as the evaporative heat loss, λE. Transpiration (evaporation), E is the amount of water vapour leaving the plant surface per unit area per unit time (kg m^{-2} s^{-1}) and λ the latent heat of vaporisation of water (2.43 x10^6 J kg^{-1}). Thus, λE has units of energy flux, W m^{-2}.

Transpiration, E is driven by the gradient of water vapour pressure between the plant surface and the air. Thus it can be written in terms of the vapour pressures at the evaporating surface and in the air

$$\lambda E = \frac{\rho c_p (e_s - e_a)}{\gamma\,(r_a + r_s)} \qquad (2.41)$$

where ρ is the density of the air (kg m^{-3}), c_p the specific heat of air at constant pressure (kJ kg^{-1} °C^{-1}), γ is the psychrometric constant (kPa °C^{-1}), e_s is the saturated water vapour pressure at surface temperature (kPa), e_a is the water vapour pressure of the surrounding air (kPa), r_a is the aerodynamic resistance (s m^{-1}) and r_s is the stomatal resistance (s m^{-1}) which depends on the number and size of the stomata through which water vapour diffuses (Althawadi and Grace 1986):

$$e_s = a\exp\left[\frac{bT_s}{c+T_s}\right]10000 \tag{2.42}$$

$$e_a = e_d\left(\frac{1-\mathrm{RH}}{100}\right) \tag{2.43}$$

where RH is the relative humidity of moist air. RH expresses the degree of saturation where air is not saturated with vapour water and is defined as the ratio of the air (dry) vapour pressure e_d (kPa) to the saturation vapour pressure e (kPa) at the same temperature:

$$\mathrm{RH} = \frac{e}{e_d \times 100} \tag{2.44}$$

$$e = e_w - \gamma\left(T_a - T_w\right) \tag{2.45}$$

where γ is the psychrometer constant (66.6 Pa K^{-1}, Jones 1996 Appendix 3) and T_w is the wet bulb temperature, the dry bulb temperature (T_d) is the air temperature T_a.

$$e_w = a\exp\left[\frac{bT_w}{c+T_w}\right]10000 \tag{2.46}$$

$$e_d = a\exp\left[\frac{bT_a}{c+T_a}\right]10000 \tag{2.47}$$

a, b and c are the empirical coefficients: a =0.061375, b =17.502, and c =240.97 (Jones 1992 pp 110).

The stomatal conductance g_s (mm s^{-1}) was calculated from the following equation (Jones 1992 pp 364):

$$g_s = \frac{E}{0.622\rho_a\left[\frac{(e_s - e_a)}{P}\right]} \tag{2.48}$$

where ra is the density of air and P is the pressure. The same equation given in mmol s^{-1} m^{-2}:

$$g_s = \frac{E}{18\times10^{-6}\left[\frac{(e_s - e_a)}{101300}\right]} \tag{2.49}$$

2.4 Metabolism (*M*)

The rate at which energy is trapped in chemical bonds by photosynthesis is less than a few percent of the energy balance for leaves and other photosynthetic tissues, and thus can usually be ignored. The oxidation of a mol of glucose in respiration releases 2809 kJ and six moles of CO_2 in the following reaction

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \tag{2.50}$$

Typical respiration rates of leaves are 1 μmol CO2 m-2 s-1, amounting to an energy flux of 0.2 W m-2, which is three orders of magnitude less than solar radiation.

However the term *M* (W m-2) becomes quantitatively important in some cases where the high respiration rates increase the temperature of the plant tissue. This is the case of thermogenic flowers, which are considered in later chapters. The metabolism pathway used by these flowers is the cyanide insentitive respiration in which no ATP is produced but the energy is released directly as heat (Laties 1982). The metabolic pathway is a variation of the citric acid cycle (Bahr, Bonner and Jr. 1973), where glycolysis predominates during thermogenesis (ap Rees, Fuller and Wright 1976).

2.5 Storage (*X*)

Very little energy is stored as heat in leaves. However some plant parts, for example tree stems, massive stems of cacti (Lewis and Nobel 1977; Nobel 1980), fruits (Patiño, Herre and Tyree 1994), flower buds, thermogenic flowers (Nobel 1991 pp 387) and perhaps flower parts of gigantic flowers are massive enough to store some heat. In these cases heat storage *X* can be defined as

$$X = \frac{\rho_t c_{pt} l \mathrm{d}T_t}{\mathrm{d}t} \tag{2.51}$$

where ρ_t is the density of plant tissue (taken as 700 kg m^{-3}), c_{p_t} is the specific heat capacity of the plant tissue (3800 J kg^{-1} K^{-1}), l is a volume to area ratio which equals the thickness for a flat petal, *d*/6 for a sphere and *d*/4 for a cylinder, where *d* is the diameter of the plant part (m), $\mathrm{d}T_t/\ \mathrm{d}t$ is the rate of change of tissue temperature with time (Jones 1992).

Table 2.3 Contains a summary of the physical properties of water, air and plant tissue used in the calculations in this thesis.

In the chapters that follow, the theory is applied in order to calculate heat production and CO_2 produced (Chapters 3 and 4); evapotranspiration and net radiation absorbed by corollas, sepals and gynoecium (Chapter 5); and geometrical relations of the sun and the flowers (Chapter 6).

Table 2.3 Nusselt numbers for air (Monteith and Unsworth 1990 pp 270-271)

Flower part (shape)		Case	Range	Nu
Corolla (flat plate)	**Free convection**	Turbulent flow	$Gr>10^5$	$0.13Gr^{0.33}$
		Laminar flow	$Gr<10^5$	$0.5Gr^{0.25}$
Bud and gynoecium (sphere)		Laminar flow	$Gr^{0.25}<220$	$2+0.54Gr^{0.25}$
		Turbulent flow		
Column (cylinder)		Laminar flow	$10^4<Gr<10^9$	$0.48Gr^{0.25}$
		Turbulent flow	$Gr>10^9$	$0.09Gr^{0.33}$
Corolla (flat plate)	**Forced convection**	Streamline flow	$Re<2x10^4$	$0.60Re^{0.5}$
		Turbulent flow	$Re>2x10^4$	$0.032Re^{0.8}$
Bud and gynoecium (sphere)		Streamline flow	$0\leq Re\leq 300$	2+0.54Re0.5
		Turbulent flow	$50\leq Re\leq 1.5x10^5$	0.34Re0.6
Column (cylinder)		*	$1<Re\leq 4$	$0.89Re^{0.33}$
		*	$4<Re\leq 40$	$0.82Re^{0.39}$

* Narrow range of Reynolds numbers

Table 2.4 Some physical roperties of air, water at 25°C, and tissue at 20°C and 100 kPa. Density, ρ, specific heat capacity, c_p, thermal conductivity, k, kinematic viscosity coefficient, ν. Subscripts: tissue, t, water, w and, air, a.

	ρ (kg m^{-3})	c_p (J kg^{-1} K^{-1})	k (W m^{-1} K^{-1})	ν (m^2 s^{-1} x 10^{-5})
Water	998.23	4187	0.607	0.089
Dry air	1.183	1012	0.026	1.55
Tissue	700	3800	0.405	

Chapter 3*

Endothermy by flowers of *Rhizanthes lowii* (Rafflesiaceae)

3.1 Introduction

Endothermy in plants was discovered over 200 years ago (Lamark 1778) and has now been documented in nine different plant families: *Cycadaceae* (Skubatz, Tang and Meeuse 1993), *Nymphaeaceae* (Prance and Arias 1975), *Aristolochiaceae, Araceae* (Knoll 1926; Meeuse 1966; Knutson 1972; Meeuse 1975; Meeuse and

Figure 3. 1 Male flower of *Rhizanthes lowii* in full bloom (Photograph by Awg Samhan b Nyawa).

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* This chapter is based on work already published (Appendix): *Oecologia* (2000) Vol 124:149-155.

Raskin 1988; Uemura, Ohkawara and Kudo 1993), *Arecaceae, Cyclanthaceae* (Schroeder 1978; Gottsberger 1990; Listabarth 1996), *Annonaceae* (Gottsberger 1989; Gottsberger 1990), *Magnoliaceae,* and *Nelumbonaceae* (Miyake 1898; Schneider and Buchanan 1980).

Several hypotheses have been presented for the ecological relevance of elevated temperatures in plants (Moodie 1976; Uemura et al. 1993): to protect the flowers from freezing (Knutson 1974; Camazine and Niklas 1984), to enhance the rate of flower development (Bermadinger-Stabentheiner and Stabentheiner 1995), to provide a direct energy reward for insect pollinators (Prance and Arias 1975; Kevan 1975; Seymour, Bartholomew and Barnhart 1983; Cooley 1995; Seymour and Schultze-Motel 1997), to increase diffusion rates of carbon dioxide and volatilisation of specific chemicals that attract pollinators (Smith and Meeuse 1966; Meeuse 1966; Chen and Meeuse 1971; Nagy, Odell and Seymour 1972; Bahr, Bonner and Jr. 1973; Meeuse 1975; Moodie 1976; Schneider and Buchanan 1980; Skubatz, Williamson, Schneider et al. 1990; Uemura et al. 1993), to enhance the growth of the pollen tube (Jewell, McKee and Richards 1994; Kudo 1995), to assure reproductive success (seed set) (Miller 1986) and to achieve mimicry of mammalian faeces and carrion to attract scavengers and carrion flies (Knutson 1972; Moodie 1976; Uemura et al. 1993).

A few species of plants have flowers that go beyond endothermy and actually regulate their temperature: *Philodendron selloum* (Nagy et al. 1972), *Xanthosoma robustum* (Meeuse and Raskin 1988), *Symplocarpus foetidus* (Knutson 1974), *Symplocarpus renifolius* (Uemura et al. 1993), and *Nelumbo nucifera* (Seymour and Schultze-Motel 1996). They maintain their internal temperature nearly constant in spite of large fluctuations in air temperature (Seymour 1997).

The genus *Rhizanthes* belongs to the wholly parasitic family *Rafflesiaceae*. It contains only two species *R. zippelii* (Blume) Spach and *R. lowii* (Beccari) Harms distributed in Borneo, Sumatra, Malaysia, Java, and Thailand (Bänziger 1995; Meijer 1997).

Rhizanthes is parasitic on the roots and near-ground stems of a few species of the vine *Tetrastigma* (Vitaceae), therefore it is an understory plant (Fig. 3.1). It has no leaves or photosynthetic tissue, stems or roots, the only vegetative parts being fine filaments that penetrate the roots and stems of the vine host. The plant produces large ephemeral flowers that are unisexual in *R. lowii* and unisexual or bisexual in *R. zippelii*.

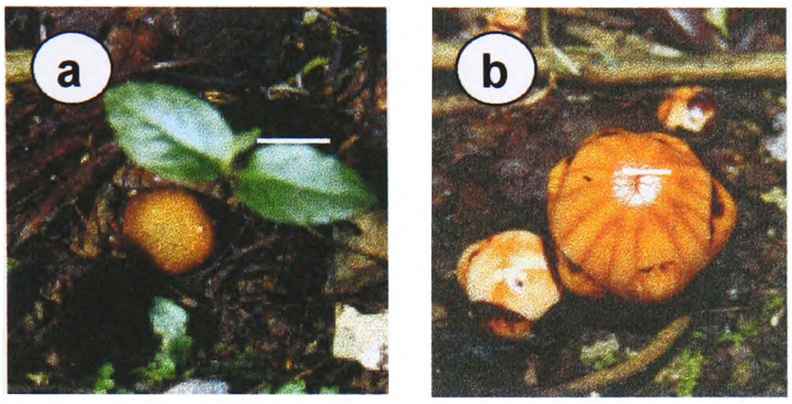


Figure 3. 2 *Rhizanthes lowii* growing in the understory of the Rain forest in Brunei, Borneo. (**a**) young bud; (**b**) young and semi-mature bud about two months before anthesis; (**c**) Flower in blossom, showing carrion flies attracted by its strong smell. White lines indicate one centimeter.

The plant is first evident as a small protuberance emerging from the roots of the host (Fig. 3.2a). After 6-7 months it takes the form of a brownish bud (Fig. 3.2b) which blooms into a large flower (Fig. 3.2c). This attracts insects of several families by producing a strong "stuffy room" and "cheese-like" odour (Bänziger 1996), or a "cadaverous" smell (some authors in Meijer and Veldkamp 1988). Of the many insect visitors, only female carrion flies of the genera *Lucilia*, *Chrysomya*, *Hypopygiopsis* effect pollination (Bänziger 1996) suggested that the hairyness of *R. zippelii* mimics mammalian pelage whereby the reproductive parts resemble a carcass, or mimics orifices or wounds of a hairy mammal, whilst producing the various volatile essences and probably CO_2 that mimic the odour of carrion, wounds or infected orifices. These stimuli induce mistaken oviposition, the hatchlings being doomed to starvation.

In the present chapter the hypothesis that *R. lowii* is an endothermic plant, as suggested by its appearance, mode of life, and pollination biology is being tested.

3.2 Methods

3.2.1 Study site. This study was conducted in the Batu Apoi Forest Reserve at the University of Brunei Darussalam Kuala Belalong Field Studies Centre (KBFSC) (115° 8' E 4° 32' N), Brunei, Borneo, S. E. Asia (Fig. 3.3). The forest at KBFSC is classified as a pristine mixed dipterocarp forest (100-800 m asl), with clay and sandstone humus-rich soils. The climate is aseasonal, with mean monthly rainfall > 100 mm for all months. The site included a small valley and the adjacent North facing slopes. (Ashton 1964) reported detailed descriptions of vegetation, climate, and soils.

3.2.2 Plant material

Rhizanthes lowii flowers are rare and in a very restricted habitat (evergreen, primary tropical rain forest, 120-160 m asl). Six clusters of *R. lowii* with 9-15 buds at all stages were distributed over an area of about 5 ha. All the clusters were parasitic on *Tetrastigma pedunculare* (Wall. ex Laws.) Planch. (Vitaceae). Individual buds were tagged to follow growth and survival. In this study, flowers that were blooming were of the same sex in each cluster. Some buds from different clusters were selected (usually the mature ones) to follow the pattern of temperature during the development of the bud until blooming and decay.

Four flower stages were classified as (1) young bud, defined as several weeks to one week before anthesis, (2) mature bud, at 7 days prior to anthesis (3) fresh

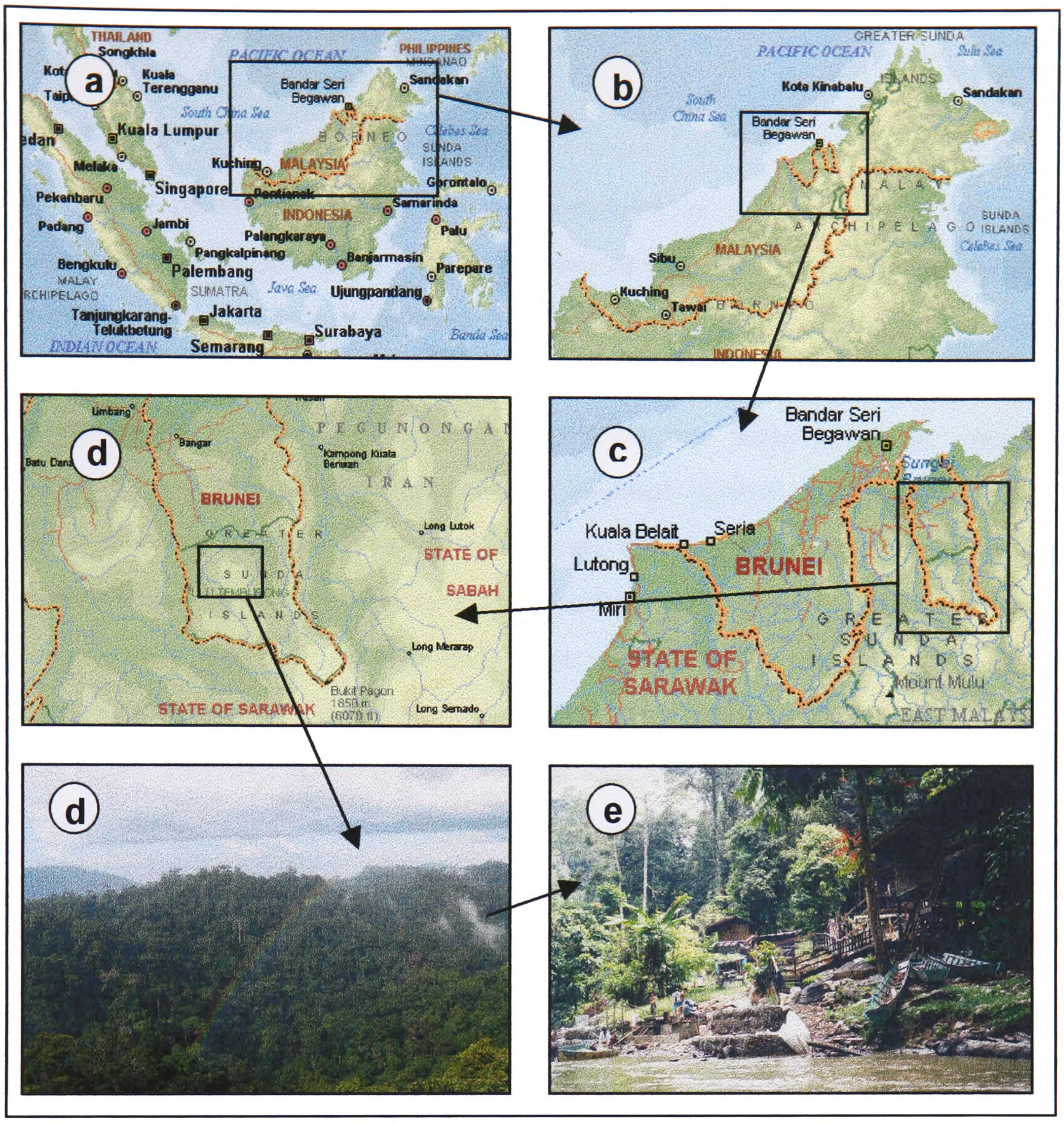


Figure 3.3 The location of the study site. (**a**) Borneo in South East Asia; (**b**) Brunei Darussalam; (**c**) Temburong district; (**d**) Batu Apoi Natural Reserve; (**e**) The forest at Kuala Belalong and; (**f**) Kuala Belalong Field Studies Centre. (Source of maps: (Expedia.com® 2000)).

flower, the first and the second days following anthesis and (4) decaying flower, from the third day following anthesis.

3.2.3 Field measurements

Flower temperature and microclimate

The diurnal pattern of temperature was measured on two female and two male flowers of *R. lowii* during the natural sequence of anthesis (before, during and after blooming), and four immature buds from different clusters. Measurements were made during three periods, from 16 October to 14 December of 1997. Copper-constantan (0.5 mm in diameter) thermocouples were used to measure temperatures. The thermocouples were calibrated against an Hg thermometer. The thermocouples were inserted in different parts of the buds to measure the temperature of: bud centre (*i*) and bud surface (*s*), (Fig. 3.4a, b); and in the open flower: ovary (female), interior (male) (*o*), anther (male) (*at*), stigmatic fascia (female) (*st*), stalk of the column (*sc*) and upper surface (*us*) (Fig. 3.4c). Soil temperature (*so*) was also recorded with an additional thermocouple 5 mm below the soil surface. Dry-bulb temperature (air temperature, T_a) and wet-bulb temperature were measured with thermocouples shaded in a custom-built forced-air hygrometer placed at 10-30 cm above the ground. Relative humidity and water vapour pressure were calculated from these temperatures (Chapter 2, equation 2.44). Wind velocity, u, at flower level at about 10 cm from the ground was measured with an omnidirectional hot-wire anemometer (model 8460--13E-V, TSI Inc., St. Paul Minnesota). Wind velocity at 30 cm above the ground was measured with a cup anemometer (Model MG2, Vector Instruments.). Net radiation, R_n, was measured with a Funk-type net radiometer (Q*7 Net Radiometer, Campbell Scientific Ltd., Leicestershire, UK). The net radiometer was placed 10 cm above the ground near the bud or open flower. Photosynthetic photon flux density (PPFD) was recorded with a quantum sensor placed just above the subject (Quantum Sensor SKP215, Skye Instruments Ltd. Llandrindod Wells, Powys, Wales, UK). (Data for wind and PPFD included in Fig 3.9).

All variables were measured every 6 s and recorded as one-minute means during the day, and every 20 s with 5-minute means during the night. Data were stored automatically on a logger (Model 21X, Campbell Scientific, Ltd., Leicestershire, UK). Later, they were plotted as average 10-minute values. To avoid any temperature effect of sunflecks on the flowers, one of the female flowers was covered with a sheet of white corrugated plastic placed 1.0 m above the ground.

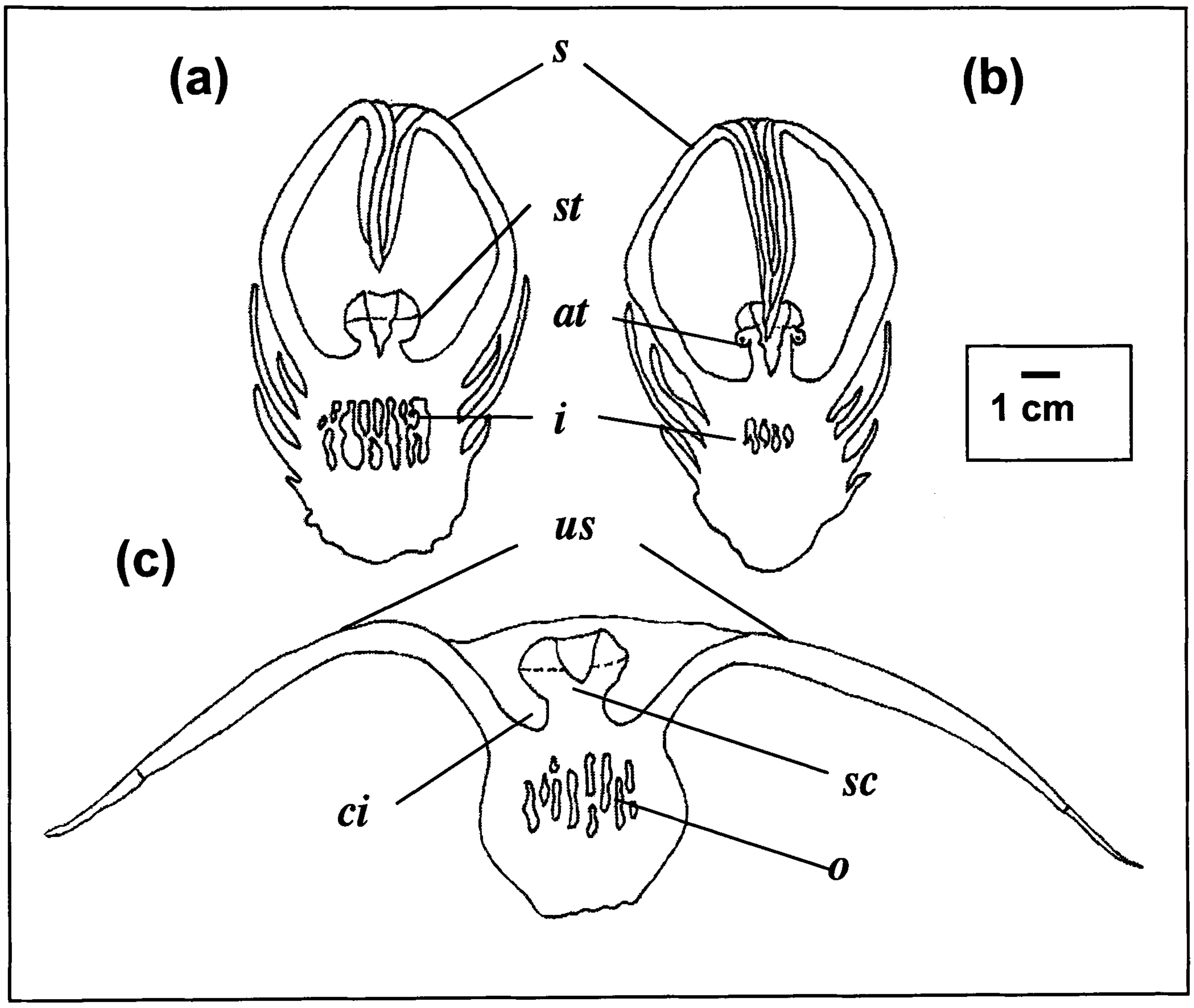


Figure 3.4 A schematic representation of a median longitudinal section through a (**a**) female bud; (**b**) male bud and; (**c**) open female flower showing the different parts. The symbols are: (*s*) bud surface, (*st*) stigmatic fascia, (*at*) anther, (*i*) bud interior, (*us*) upper surface of the petal, (*ci*) circumambulator, (*sc*) stalk of the column and, (*o*) ovary (female).

3.3 Results

Flower temperature and microclimate

Diurnal changes in temperature were studied for several days on four young buds, two male and two female flowers.

Bud Temperature

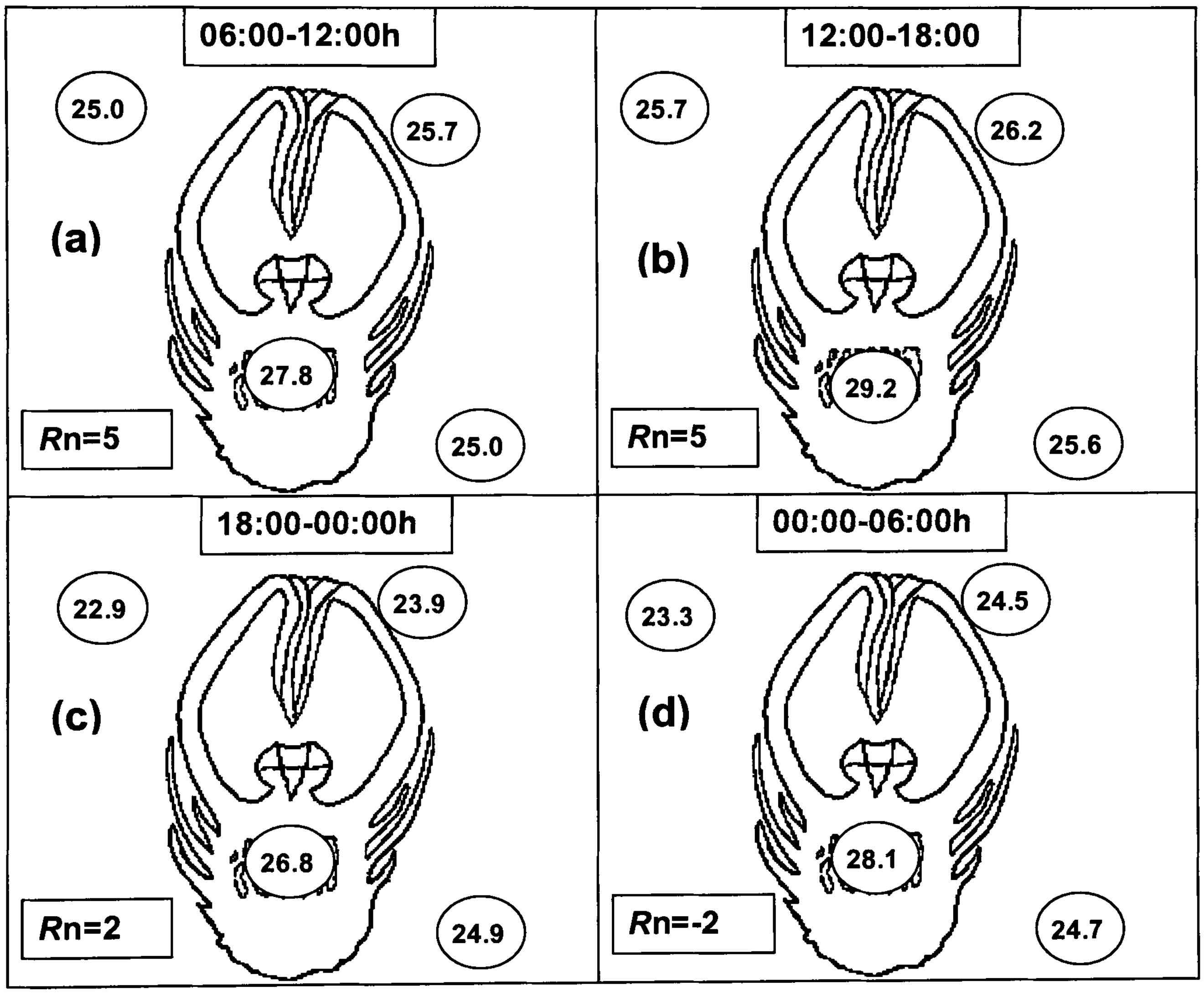
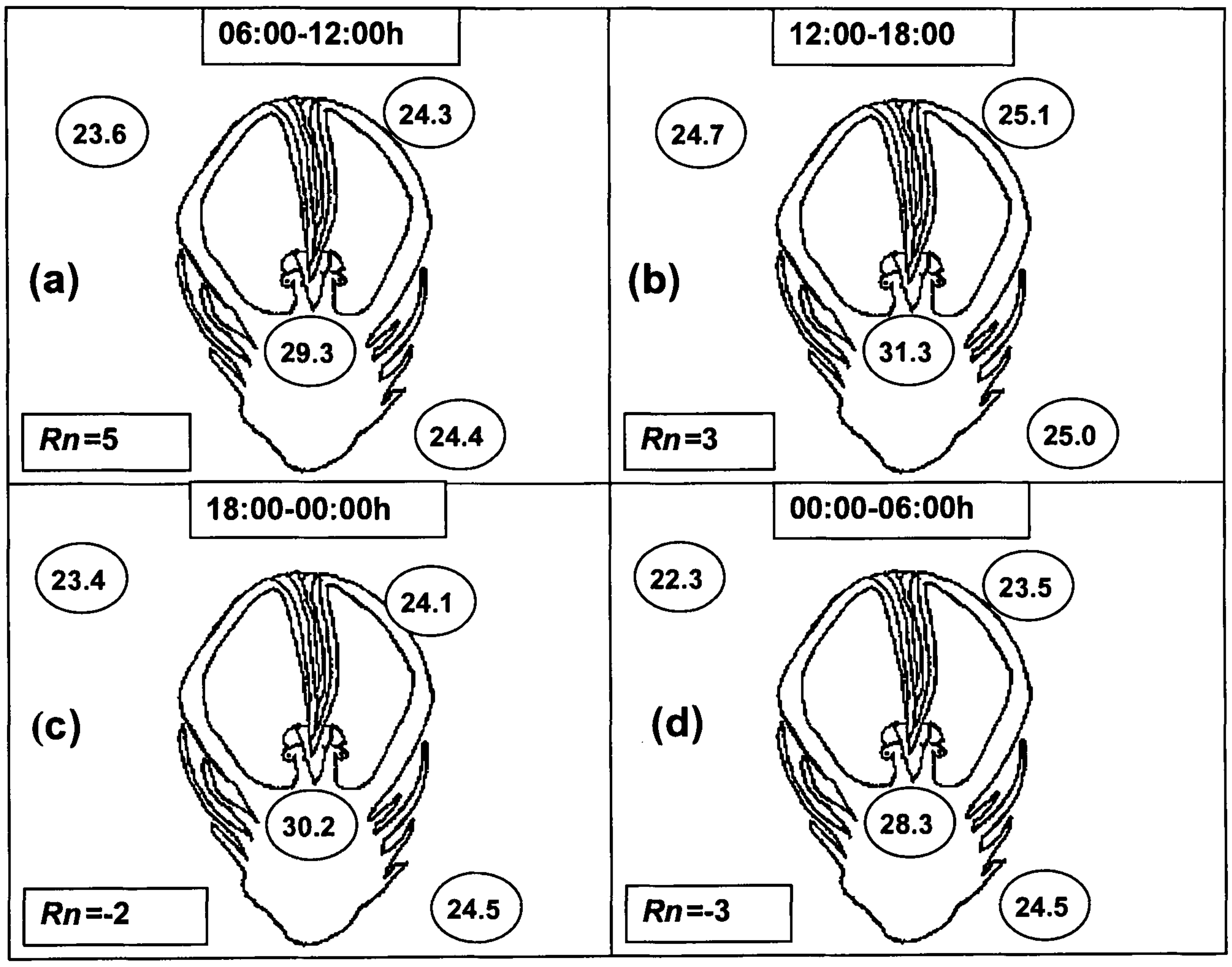


Figure 3.5 Schematic representation of tissue temperature for a young female bud during the day. The day is divided into four periods of six hours each. Each temperature value represents the period mean temperature over 2-day. (**a**) from 06:00 to 12:00 h; (**b**) from 12:00 to 18:00 h; (**c**) from 18:00 to 24:00 h; (**d**) from 24:00 to 06:00 h. The numbers inside ovals are the mean values of temperature in °C. Net radiation (R_n) is given in W m^{-2}.

The mean temperature of different parts of both, female and male buds over two days are shown in Figures 3.5 and 3.6 grouping data according to time of day. The female bud was younger (about two months before anthesis) than the male bud (two days before anthesis) consequently the temperatures of the male bud (Fig. 3.6) seem to be higher than the temperatures of the female bud (Fig. 3.5). The interior of the buds were consistently warmer than the air during the night and the day. For both, female and male buds the highest temperatures were reached in the afternoon (12:00 - 18:00, Fig. 3.5b and 3.6b). The female bud was cooler in the evening (18:00 - 00:00, Fig. 3.5c) whilst the male bud was cooler in the night before dawn (00:00 - 06:00, Fig. 3.6d). Mean net radiation did not exceed 5.0 W



Figures 3.6 Schematic representation of tissue temperature for a mature male bud during the day. The day is divided into four periods of six hours each. Each temperature value represents the period mean temperature over 2-day. (**a**) from 06:00 to 12:00 h; (**b**) from 12:00 to 18:00 h; (**c**) from 18:00 to 24:00 h; (**d**) from 24:00 to 06:00 h. The numbers inside ovals are the mean values of temperature in °C. Net radiation (R_n) is given in W m^{-2}.

m^{-2}. The mean of air temperature did not vary much; from 22.9 °C to 25.7°C for the female bud and from 22.3 °C to 24.7 for the male bud. Soil temperature was fairly constant for both female and male buds. The mean temperature of different parts of the female fresh flower over two days is shown in Figure 3.7 and of the male fresh flower is shown in Figure 3.8. In the female flower all tissue temperatures were consistently warmer than the air, often by more than 6 K in the centre of the floral structure. In the male flower the interior was frequently more than 7 K. The net radiation was always low, in the range +2.6 to -3.5 W m^{-2} for the female flower. For the male flower the net radiation was in the range of -3.4

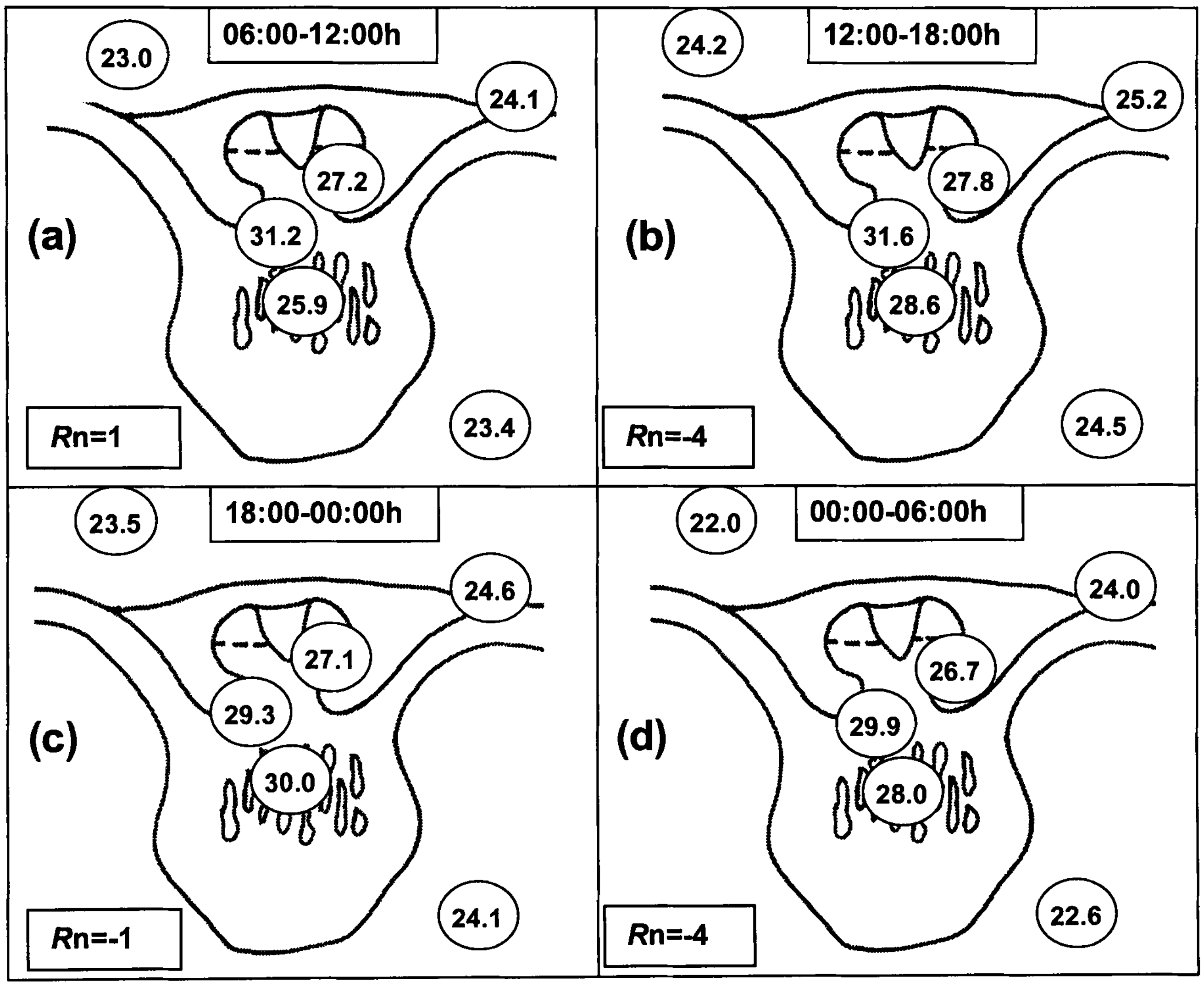


Figure 3.7 Schematic representation of tissue temperature for a female flower during the day. The day is divided into periods of six hours each. Each temperature value represents the period mean temperature over 2-day. (**a**) from 06:00 to 12:00 h; (**b**) from 12:00 to 18:00 h; (**c**) from 18:00 to 24:00 h; (**d**) from 24:00 to 06:00 h. The numbers inside the ovals are the mean values of temperature in °C. Net radiation is given in W m^{-2}.

and +5.5 W m^{-2}. The temperature of the stalk of the column (the structure that limits the region visited by pollinating insects, (see Fig. 3.4c) did not vary much, being 30 ± 1 °C in the female flower whilst the ambient temperature varied about 23 ± 1 °C and the soil varied about 22.5 ± 2 °C. For the male flower the ambient temperature varied by about around 24 ± 1 °C and the soil varied about 24.5 ± 1 °C. The ovary of the female flower was predominantly warm during the evening (18:00 - 00:00, Fig. 3.7c) whilst in the male flower the interior was warmer mainly during the afternoon (12:00 - 18:00, Fig. 3.8b). The stigmatic fascia was almost constant for the four periods and the anther varied only 1 °C. The surface temperature of the female flower fluctuated from 25 °C to 28 °C and of the male

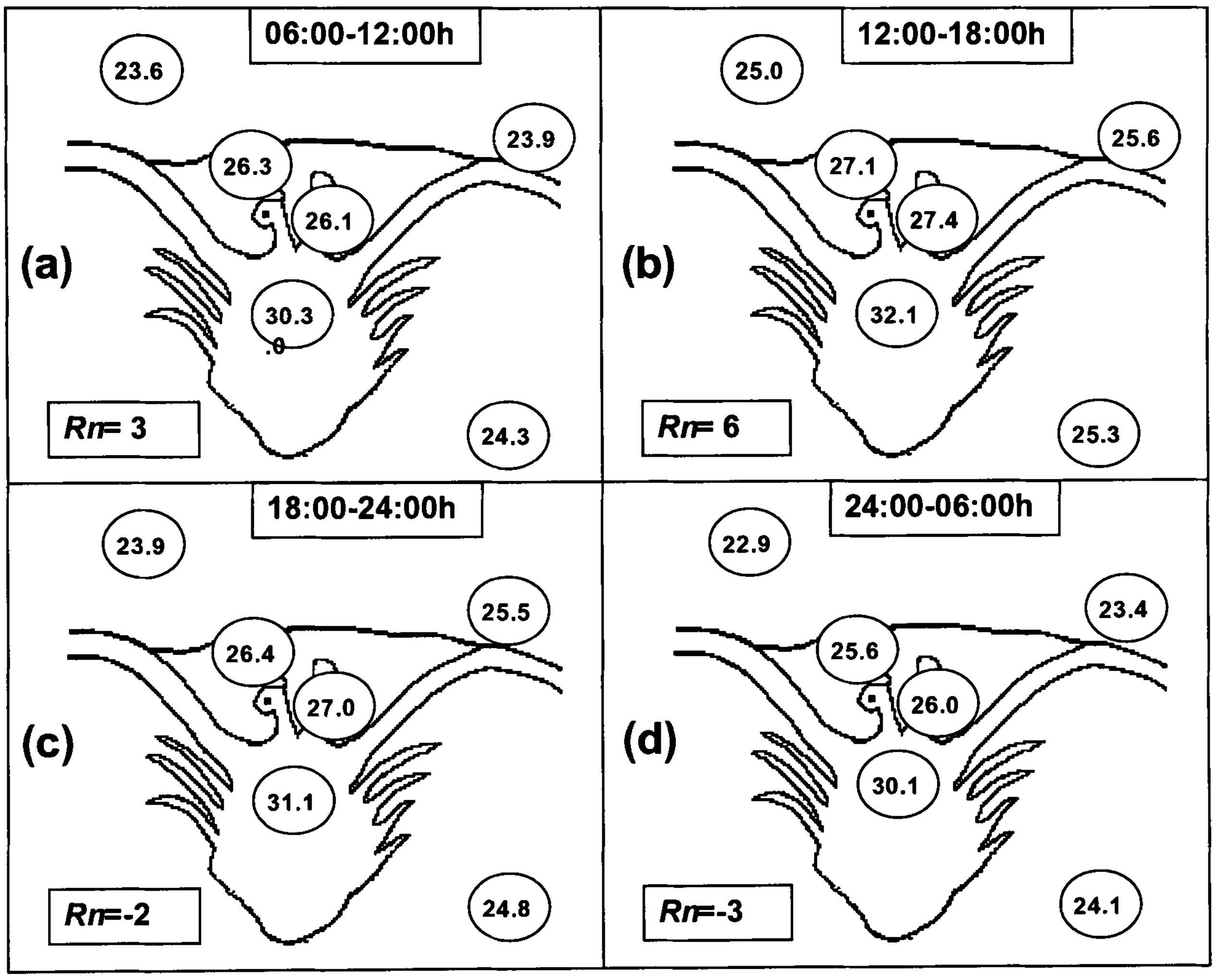


Figure 3.8 Schematic representation of tissue temperature for a male flower during the day. The day is divided into four periods of six hours each. Each temperature value represents the period mean temperature over 2-day. (**a**) from 06:00 to 12:00 h; (**b**) from 12:00 to 18:00 h; (**c**) from 18:00 to 24:00 h; (**d**) from 24:00 to 06:00 h. The numbers inside ovals are the mean values of temperature in °C. Net radiation (R_n) is given in W m^{-2}.

lower fluctuated from 23 °C to 26 °C and followed the diurnal pattern of ambient temperature.

The term "excess temperature" will hereafter be used to indicate the extent to which the tissue is warmer than the air and will be expressed in K.

Microclimate and excess temperature.

Female flowers. Fig. 3.9a-e shows a representative 10-day course of excess temperature and microclimatic variables for the young bud and the female flower in November 1997.

One young bud and one mature bud were studied simultaneously for almost 10 days until premature abscission of the young bud on day 7 (Fig. 3.9a). The mature bud opened into a flower in the early morning of day 7 (Fig. 3.9b). The interior of the young bud (line number 1, Fig. 3.9 a) was 1 K above T_a at the beginning of the study period and 4 K before abscission whilst the surface (line number 4, Fig. 3.9 a) never exceeded 1 K. The excess temperature of the internal tissue increased every day in the late afternoon, when rain usually occurred. The diameter of the bud increased at a rate of about 3 mm per day (from 25 to 40 mm). Fig. 3.9 b shows the diameter of the mature female bud which was about 60 mm at six days before anthesis, increasing by approximately 3 mm per day, and reaching 75 mm on the day before anthesis (day 6 Fig. 3.9b).

During the period of measurements, as the bud increased in size the excess temperature of the stalk of the column rose over a five-day period (days 2-6, Fig. 3.9b) with a tendency for maximal to occur in the late afternoon and early in the morning prior to anthesis. The surface temperature of the mature bud (line 4, Fig. 3.9b) remained fairly constant at about 3 K above the air during the 6 days period prior to anthesis, increasing on the night of anthesis (day 7, Fig. 3.9b) to the maximum excess temperature of 4.1 K. This suggests a high rate of metabolic

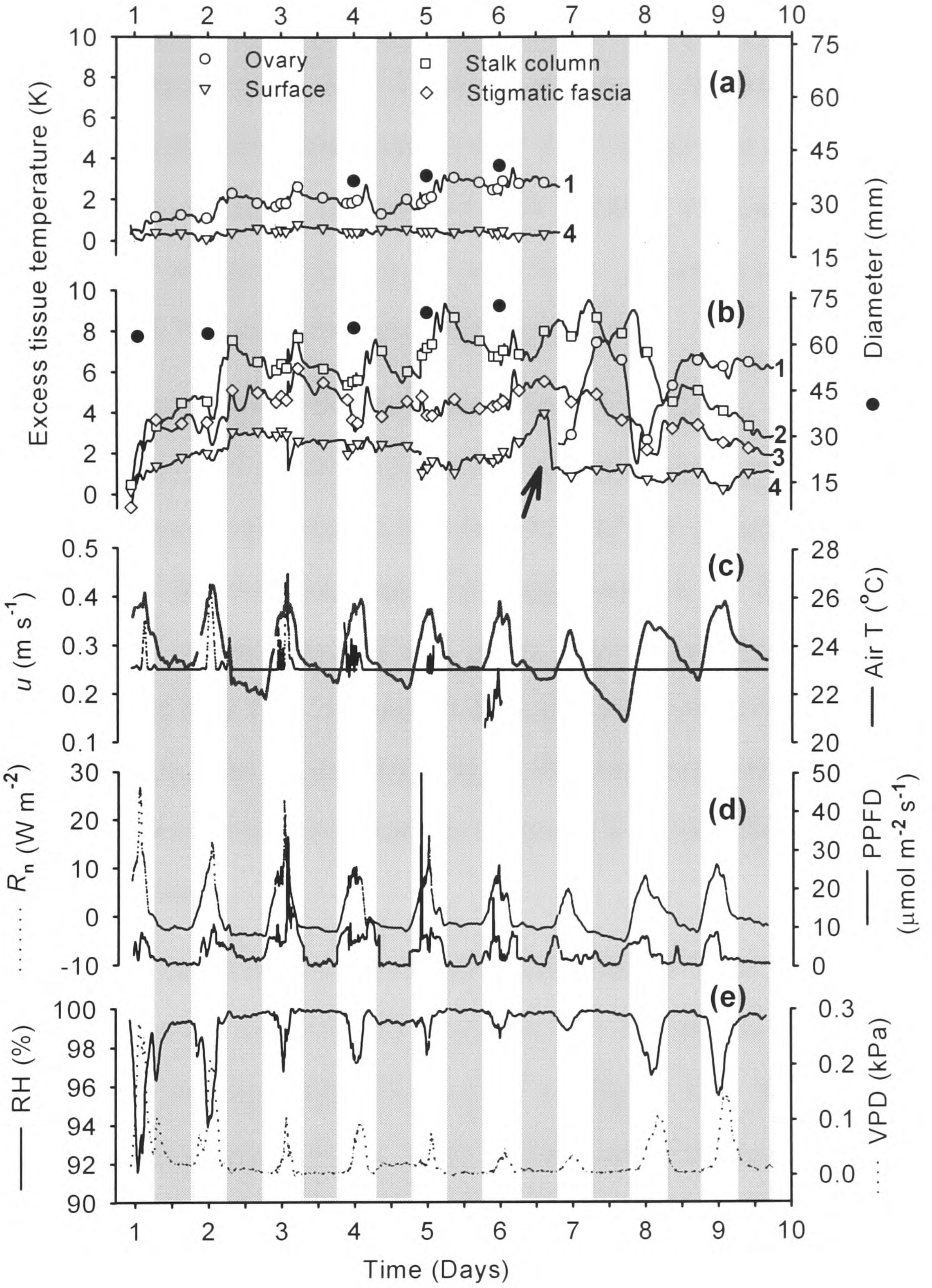


Figure 3.9 Excess temperature and the microclimate of bud and female flower parts of *R. lowii* from the 17th to 26 November 1997. Nights are indicated by grey shadows, closed circles indicate bud diameter the arrow indicates anthesis. (**a**) young bud, lines and symbols indicate:, interior (line 1, circle) and surface (line 4, triangle); (**b**) Excess temperature courses for flower parts:- ovary (line 1, circle), stalk of the column (line 2,square), stigmatic fascia (line 3, diamond), and surface (line 4, triangle). The figure follows the sequence of anthesis: mature bud (day 1-6), anthesis (arrow), fresh flower (day 7-8) and decaying flower (day 9-10); (**c**) net radiation and PPFD; (**d**) wind velocity measured with a hot wire anemometer (continuous line) and measured with a cup anemometer (dotted line); and air temperature (bold line); (**e**) relative humidity and flower surface-to-air vapour pressure deficit.

activity during anthesis. The surface temperature of the flower (day 7-10, Fig. 3.9b) was close to air temperature with a maximum excess of about 1.5 K.

The ovary temperature (line 1, Fig. 3.9b) was measured from the day of anthesis, showing its maximum excess temperature of 7.8 K on the first night of blooming, entering a cooler period on the second day, and reaching a minimum excess of 1.6 K, increasing again on the second night to 7.2 K. Thereafter it was maintained fairly constant at 6.5 K during the decaying period.

The maximum excess temperature was as high as 9.6 K (day 7, Fig. 3.9b) and was achieved in the stalk of the column on the first day of blooming (line 2, Fig. 3.9b). Three peaks of excess were observed in the stalk of the column during the fresh flower stage: one in the morning, one in the afternoon on the first day and one in the morning on the second day. These peaks were coincident with the time of maximum activity of flies (10-11h and 17-18h) (personal observation). The excess of the stigmatic fascia (line 3, day 3-6, Fig. 3.9b) was particularly constant during the mature bud stage reaching a maximum excess of 6 K and started to decline after the first day of anthesis.

The average of net radiation during the day was about 8 W m^{-2} and never exceed 30 W m^{-2}, PPFD was normally 13 μmol m^{-2} s^{-1} during the day, with a few sunflecks at midday not exceeding 50 μmol m^{-2} s^{-1} (Fig. 3.9c). Wind velocity at the flower level never exceeded 0.5 m s^{-1} and air temperature fluctuated between 21 °C and 26 °C (Fig. 3.9d). The relative humidity was very high and VPD was extremely low during the whole period (Fig. 3.9e).

Male flowers. Figure 10 shows the excess temperature of different parts of one mature bud developing into a flower (Fig. 3.10a) and one fresh flower turning into a decaying flower (Fig. 3.10b). Temperatures were measured on individuals from the same cluster at the same time for a 7-day and 5-day period respectively. The diameter of the bud increased at a rate of about 1.3 mm per day (from 63.0 to 69.5 mm during this period).

The excess temperature of the mature bud (line 1, Fig. 3.10b) increased from about 1.0 °C at the beginning of the study period to 7.0 °C two days before anthesis.

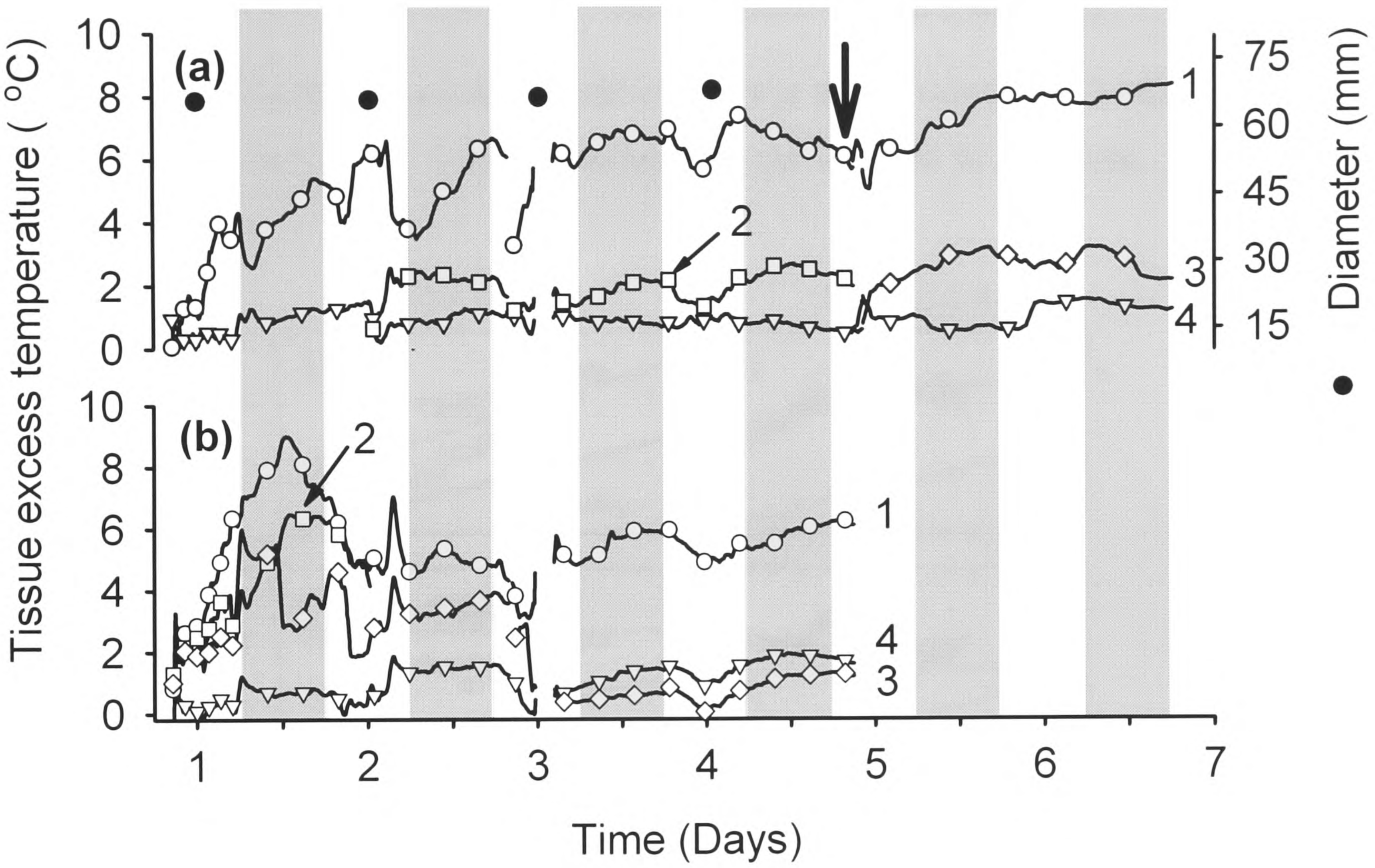


Figure 3.10 Excess temperature of bud and male flower parts. (**a**) mature bud (day 1-4), anthesis (arrow) and, fresh flower (day 5-6); (**b**) fresh flower (day 1-2) and decaying flower (day 3-5). Nights are indicated by grey shadows, closed circles indicate bud diameter, common symbols indicate: column (circle), annulus (square), anther (diamond), bud surface before anthesis (triangle) and, abaxial surface of the petal during blooming (triangle). To simplify the plot, only a sample of the data points are attached to the plotted curves.

One day before anthesis the thermocouple inside the column was relocated (see Fig. 3.10a). During the blooming period, the excess temperature was nearly constant with a maximum value of 7.8 °C. For the second flower (Fig. 3.10b), in which measurement of the column temperature started on the day of anthesis, the excess temperature of the column was as high as 9.0 °C on the first night of blooming (line 1, Fig. 3.10b), before a decline on the second day. This pattern was also observed on the female flower as explained above.

The excess temperature of the annulus in this mature bud was between 0.7 °C and 2.7 °C, increasing in late afternoon, remaining constant during the night, and decreasing during the day, reaching minimum values at noon (line 2, Fig. 3.10a).

The pattern of excess temperature of the anther was nearly constant at 3 °C for the first flower (Line 3, Fig. 3.10a) showing a slight increase in the night. In the

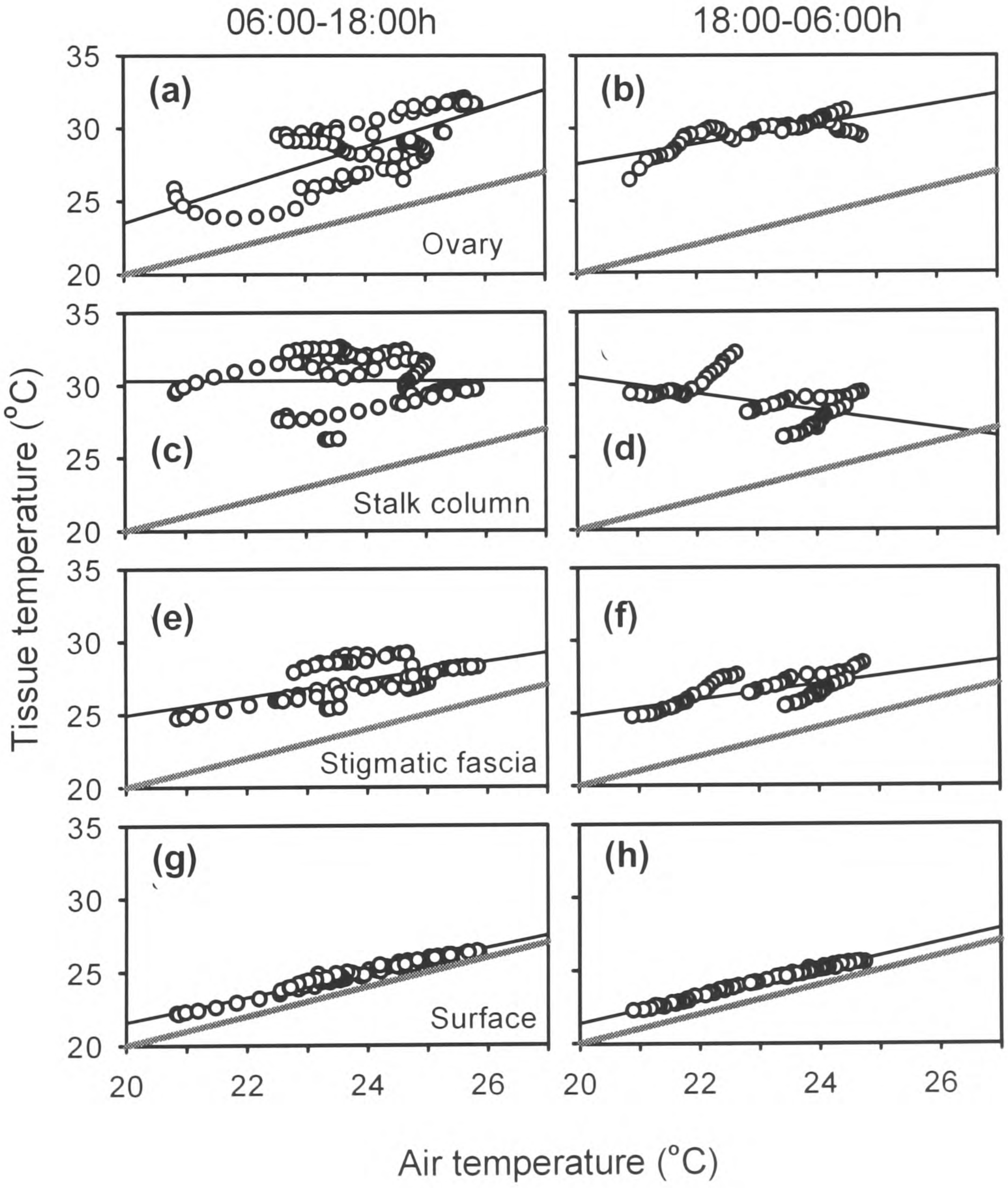


Figure 3.11 Relationship between air temperature and tissue temperatures of a fresh female flower. To simplify the plots data were plotted every five to seven readings of the original data. **(a-g)** day-time; **(b-h)** night-time; **(a-b)** ovary; **(c-d)** stalk of the column; **(e-f)** stigmatic fascia; and **(g-h)** surface. Bold line indicates one-to-one relationships of air temperature. All internal parts are consistently warmer than the air and the stalk of the column shows evidence of thermoregulation.

second flower, the pattern is variable with fluctuations during the blooming period, reaching a maximum value of 5.5 °C on the first night of blooming. In general, the stigmatic fascia in females flowers were more endothermic than the anthers for male flowers.

The surface temperature for the mature bud was close to ambient, showing an excess temperature of 1.0 ± 0.3 °C during the bud period and increasing to 1.7 °C

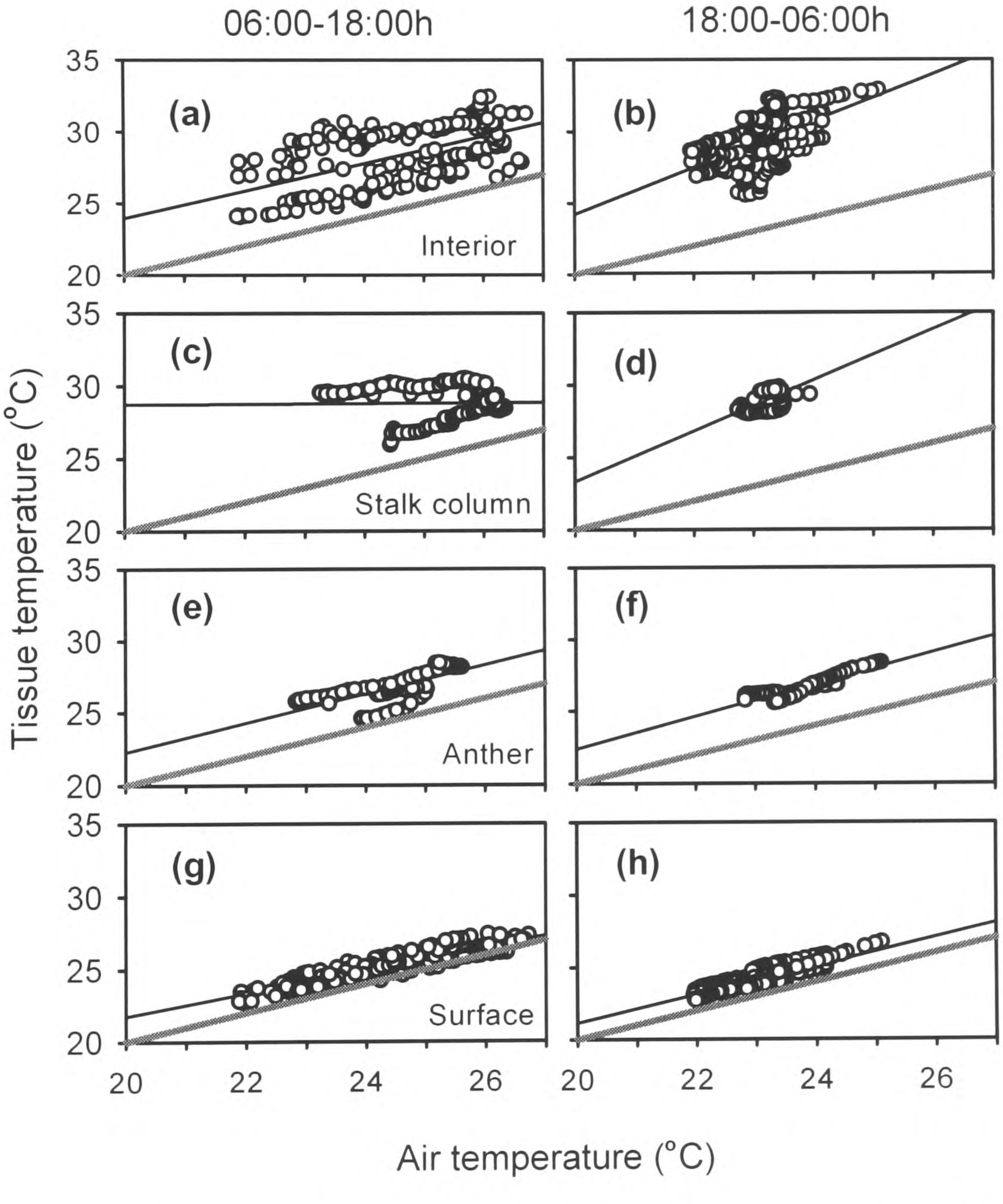


Figure 3.12 Relationship between air temperature and tissue temperatures of a fresh male flower. To simplify the plots data are plotted by five to seven readings of the original data. The left-hand (**a-g**) shows day-time, and the right-hand (**b-h**) shows night-time; (**a-b**), Interior, (**c-d**), Stalk of the column, (**e-f**), Stigmatic fascia, and (**g-h**) Surface. Bold line indicates one-to-one relationships of air temperature. All internal parts are consistently warmer than the air and the stalk of the column shows evidence of thermoregulation.

on the first day of blooming (day 5, Fig. 3.10a).

To explore the relation between tissue and air temperature, and to test the hypothesis of thermoregulation, the tissue and air temperatures were plotted as linear regressions for female (Fig. 3.11) and male (Fig. 3.12) flowers. It is evident that the slope of the relationship is often very shallow, and that in the case of the stalk of the column the tissue temperature hardly changed despite significant variation in air temperature. This indicates thermoregulation.

There is no obvious difference between the patterns shown in the female versus the

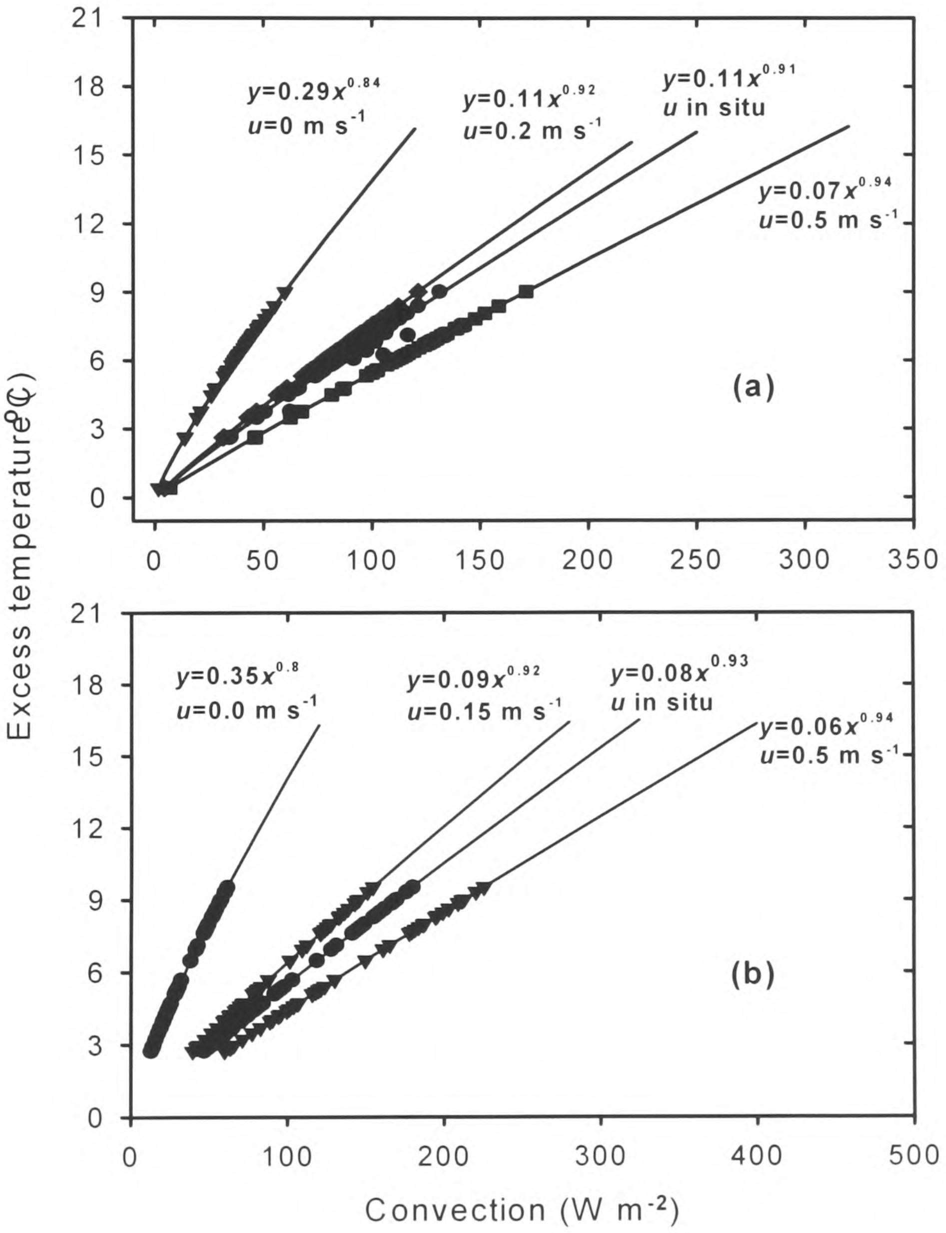


Figure 3.13 Calculation of the heat supply needed to rise the temperature of the flowers to the range observed for different wind speeds (u, m s^{-1}) near the surface of the flower. The symbols indicate the calculated heat supply using the observed temperature of the tissue, the lines represent the fitted curve using the correspondent equations. (**a**) bud interior; (**b**) stalk of the column.

male flowers (Fig. 3.11 and Fig. 3.12). The highest temperature is always for the internal tissues whilst the surface temperature is closely coupled to air temperatures.

There was a lack of correlation between net radiation (measured with a Funk-type net radiometer) and excess temperature of bud and flower parts during the sequence of blooming (data not shown). This lack of correlation indicates that the high temperature detected in the flowers is endogenous, not being dependent on the radiation load.

Direct measurements of oxygen consumption are normally used to estimate the metabolic heat production in thermogenic flowers. Since it was not possible to

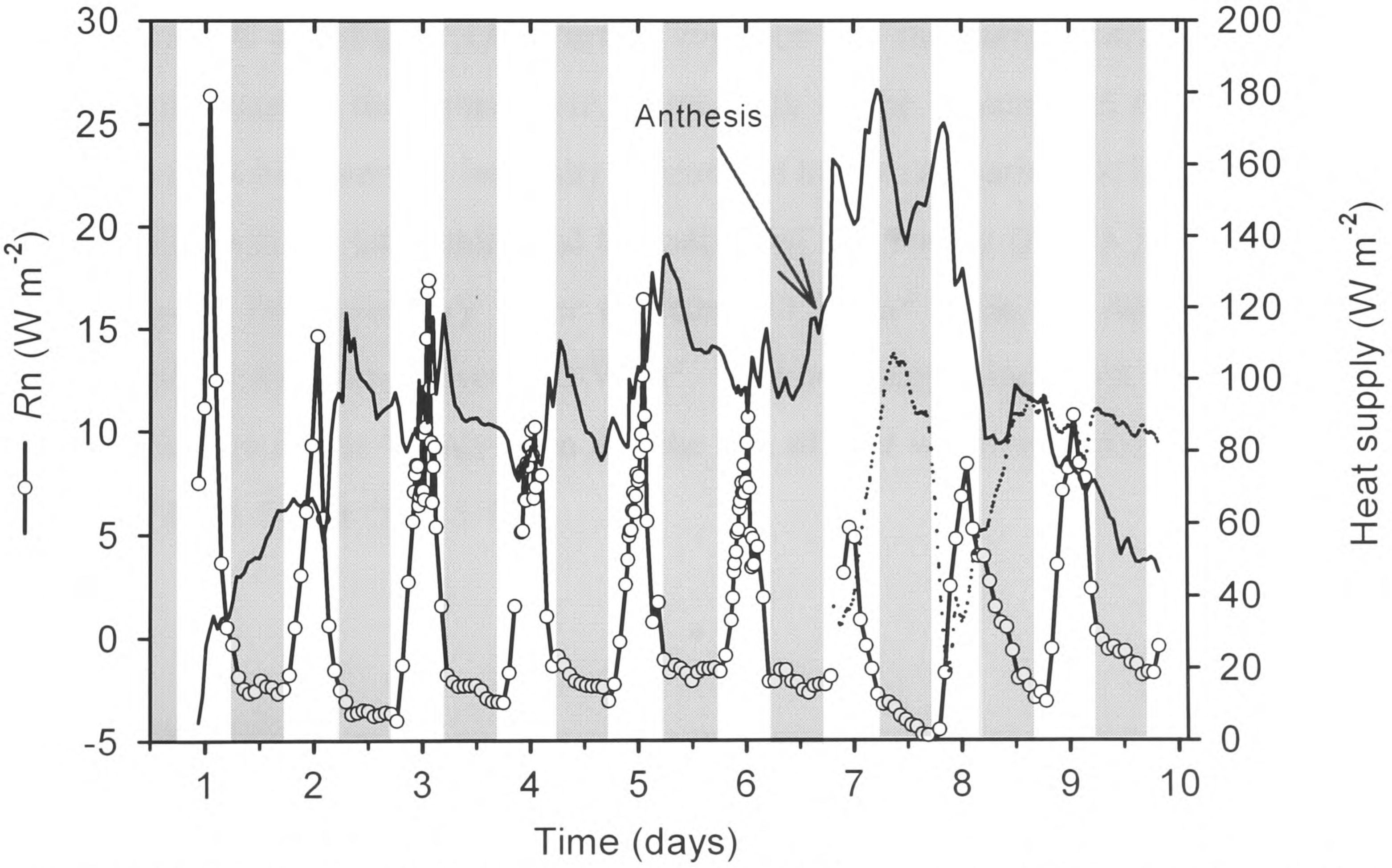


Figure 3.14 Calculated metabolic heat produced by a female flower compared to the net radiation in the understory for a period of ten days. This is the heat required to produce the observed elevation in temperature of the flower. Open symbols indicate the measured net radiation over the flower, the continuous line indicates the stalk of the column and the dotted line indicates the heat produced in the ovary.

perform direct measurements of respiration in the field to estimate the heat supply required to rise the tissue temperature up to 9.6 K above the ambient, it was calculated using an energy budget approach. In this approach, the heat transfer was modelled as a combination of forced and free convection (Chapter 2, equations 2.22 - 2.37), it was assumed that the surface was completely dry, since no data for the stomatal conductance was available. For the buds, a spherical surface was assumed and for the open flower the stalk of the column and the ovary were treated as cylinders. Physical properties of the flower material (conductivity, specific heat capacity) were assumed to be equal to other plant tissues.

According to the model the heat supply necessary to produce an excess temperature 6-7 K inside a female mature bud (7 days prior to anthesis) with a dry surface would be about 100 W m^{-2} (Fig. 3.13a). When the flower opens the heat supply to keep an excess temperature of 6-7 K in the stalk of the column would need to be about 130 W m^{-2} (Fig. 3.13b).Further evidence that the energy required for the flower to increase the temperature of the stalk of the column and the ovary or interior must be generated internally is provided by the comparison of the measured net radiation in the understory and the calculated heat supply (Fig. 3.14). The net radiation in the understory never exceeded 30 W m^{-2} while the calculated heat produced by the flower gives 180 W m^{-2}. The heat supply increased substantially when the flower was freshly open and the rate of heat was maintained for the two days of fresh flower (Fig. 3.14).

3.4 Discussion

It has been shown that *R. lowii* is an endothermic plant in which buds and blooming flowers constantly maintain their internal temperature several degrees above ambient. Endothermic activity was detected several weeks before anthesis, in young buds, progressively increased to reach its maximum two days before anthesis and persisted for some days after the flower had decayed.

One or two days before anthesis and during the two days after anthesis the flower kept its internal temperature almost constant between 30 °C and 33 °C when the air temperature fluctuated between 21 °C and 26 °C. The relative constancy of internal flower temperature suggests thermoregulation by the flower. It has been demonstrated that many flowers increase their temperature above the ambient as a consequence of endothermy, the heat being produced by the cyanide insensitive respiration (Meeuse and Raskin 1988; Skubatz et al. 1990). However, thermoregulation is unusual, implying the possession of a control mechanism. The support for thermoregulation in this study is that the flower maintains a relative constant internal temperature regardless of the ambient temperature and the net radiation of the flower.

By comparing the 1:1 regression lines in Figs. 3.10 and 3.11 it is concluded that for either female and male flowers the most endothermic and thermoregulatory part was the stalk of the column. The stalk of the column is located in the centre of the circumambulator (Fig. 3.3c) which is the area where the flies enter and "by coincidence" their dorsal regions touch the stigma and anthers in female and male flowers and effect pollination. Consequently, endothermy and thermoregulation in *R. lowii* seems to be related with two phases of the pollination process. First, endothermy may be connected to the volatilisation of odorous chemicals that contribute to the long-distance attraction of the pollinators (manuscript in preparation). On the other hand, it seems to be connected to the respiratory release of CO_2 which, in combination with other volatile chemicals produced by the flower, may stimulate oviposition. Such a synergistic effect by CO_2 has been shown in the fly *Lucilia cuprina* (Wiedemann) (Barton Brown 1979) although this species lays on live sheep which emanates CO_2 while the pollinators of *Rhizanthes* are carrion breeders. Oviposition may indirectly enhance pollination by keeping the flies searching longer for suitable sites to lay, one of the best being the circumambulator, entering and leaving of which is a precondition for pollination.

Endothermy was detected in young buds and decaying flowers as well as in mature flowers. To what extent is endothermy in the young buds an adaptation to survival? Two possible alternatives are suggested. One is that the "machinery" for producing heat is formed early in life, and production of heat is then incidental because it is of no cost for the plant itself (being a parasite it obtains its energy source from its host), although the host has a limited source of energy. Therefore, it may not have implications for survival.

The second alternative is that endothermy of the buds assists the release of defence volatiles. This seems less likely, as in a one-year study at Kuala Belalong bud mortality was as high as 90% and was attributed to fungal or bacterial attack (Awg. Samhan Nyawa). Moreover, during 1998 the few buds observed were eaten by bearded pig (*Sus barbatus*) (Patiño, personal observations).

The results suggest that there are no significant differences in the patterns of endothermy and thermoregulation between female and male flowers.

For both female and male flowers, the ovary and interior respectively became the most endothermic part after anthesis. The continuing endothermy after the flowers decayed in the case of the female flowers, may help to promote the growth of the pollen tube and development of the seeds.

Detailed studies by Bänziger (1996) on the pollination ecology of *R. zippelii* revealed that this is a sapromyophilous flower in which pollination is based mainly on brood-site deception, and only to a small extent on food reward (sugars produced at the nectar pads). The same pollination syndrome was observed in *R. lowii*. It seems that the genus *Rhizanthes* does not offer heat as a reward (because the air temperatures in the forest are sufficient for normal flight). Moreover, the food reward appears to be of secondary importance to the pollinator flies.

There may be some common characteristics among endothermic plants, but *Rhizanthes* seems not to comply. For example most thermogenic flowers are monoecious and protogynous (the maturation of female flowers before those of male), while the flowers in this study were all unisexual (Seymour and Schultze-Motel 1997). It is also suggested that endothermy is present only in ancient families of flowering plants (Seymour and Schultze-Motel 1997). It is unclear whether the *Rafflesiaceae* are among the most primitive angiosperms as parasitism involves dramatic morphological and molecular changes (Schoen and Ashman 1995). It has not been settled whether it belongs in or near the Aristolochiales or whether it is a member of the Rosidae (Stanton, Snow and Handel 1986; Röse, Manukian, Heath et al. 1996; Rascher, Blasius, Beck et al. 1998). In any case, it seems that endothermy in flowers is a homoplastic character present in different taxonomic groups not necessarily congruent with a similar morphological organisation.

3.5 Summary

1. *Rhizanthes lowii* (Beccari) Harms (Rafflesiaceae) is a parasitic plant that grows in the understory of the rain forest in South East Asia. This plant does not have leaves, stems, or photosynthetic tissue and is characterised by the emission of a strong odour that attracts the natural pollinators, carrion flies.

2. Flowers that volatilise odorous compounds and attract carrion flies, beetles and other insects are often thermogenic. There was evidence of both thermogenesis and thermoregulation in *R. lowii* from microclimate and tissue temperatures measured during different stages of flower development in *R. lowii*, in natural conditions in Brunei, Borneo.

3. Endothermy was detected in young and mature buds as well as in blooming flowers and even in decaying tissues three or more days after blooming. Tissue temperatures were maintained at 7 - 9 K above air temperature, in both female and male flowers, at all stages of floral development.

Chapter 4

Is *Rafflesia* an endothermic flower?

4.1 Introduction

The genus *Rafflesia* R. Br. belongs to the wholly parasitic family Rafflesiaceae which comprises 16 known species (Fig. 4.1). It is known to be the largest flower of the plant kingdom (13 - 107 cm diameter, (Meijer 1984) and also one of the rarest, being threatened with extinction as a consequence of habitat destruction (Meijer 1985; Beaman et al 1988; Ismail 1988; Salleh 1991; Nais and Wilcock 1998). The genus is distributed from well north of Kra Isthmus of Thailand through W. Malaysia and the Philippines, Borneo, Sumatra and Java (Bänziger 1991; Salleh 1991; Meijer 1997). *Rafflesia* is an understory plant, which is first

Figure 4.1 Female flower of *Rafflesia tuan-mudae*. An endemic species of Sarawak, Malaysia

Figure 4.2 Sequence of blooming of *Rafflesia tuan-mudae*. (**a**) young buds, about three and four months old; (**b**) mature bud, about 9 months old; (**c**) opening bud, after nine months growth; (**d**) female flower almost in full bloom

evident as a small protuberance emerging from the roots or near-ground stems of a few species of the vine *Tetrastigma* (Vitaceae). After 3 –12 months it takes the form of a brownish cabbage (Fig. 4.2a & 4.2b), which blooms into an ephemeral flower (Fig. 4.2c & 4.2d) but lacks leaves or photosynthetic tissue, stems or roots, the only vegetative parts are fine filaments that penetrate the tissue of the vine host. The flowers are unisexual (Beaman et al 1988; Bänziger 1991) and by looking and smelling like rotten flesh or a festering sore, they attract several species of carrion or blowflies of the genus *Lucilia* and *Chrysomya* (Calliphoridae) that pollinate them (Beaman et al 1988; Bänziger 1991). If pollinated after 6-9 months the structure below the column that hold the ovary of the female flower becomes the fruit, containing many thousands of miniature seeds that are more likely to be dispersed by small mammals such as squirrels and treeshrews (Meijer 1985; Emmons et al 1991). How the seeds penetrate the host is still unclear.

In the preceding chapter it was shown that the related species *Rhizanthes lowii* (Beccari) Harms (Rafflesiaceae) is a strongly endothermic flower, which exhibits weak thermoregulation. Endothermy in plants has been associated with an increase in cyanide-insensitive respiration, an alternative pathway of the respiratory electron transport system in the citric acid cycle that generates heat without producing adenosine triphosphate (ATP) (Bahr et al 1973; Meeuse 1975; McCaig and Hill 1977; McNulty and Cummins 1987; Raskin et al 1989; Kapulnik et al 1992). This process causes production and diffusion of copious amounts of carbon dioxide (Buggeln et al 1971) and volatile compounds (Meeuse 1966; Meeuse 1975) that may act as attractants of fly and beetle pollinators. The results of the preceding chapter suggested that carbon dioxide, when combined with other volatile compounds, increase the probability of pollination of *Rhizanthes lowii* (Patiño et al 2000).

Although there are morphological and functional differences, *Rafflesia* and *Rhizanthes* share many characteristics. They are sympatric in many areas, and both are understory species totally parasitic on a few but different species *Tetrastigma ssp.* lianas. Both are characterised by producing smelly volatiles and are pollinated only by carrion or blowflies. In this chapter the hypothesis that the flowers of *Rafflesia tuan-mudae* Beccari have similar endothermy patterns to the one described for *Rhizanthes lowii* (chapter 3) is tested and its possible role in the pollination syndrome is discussed.

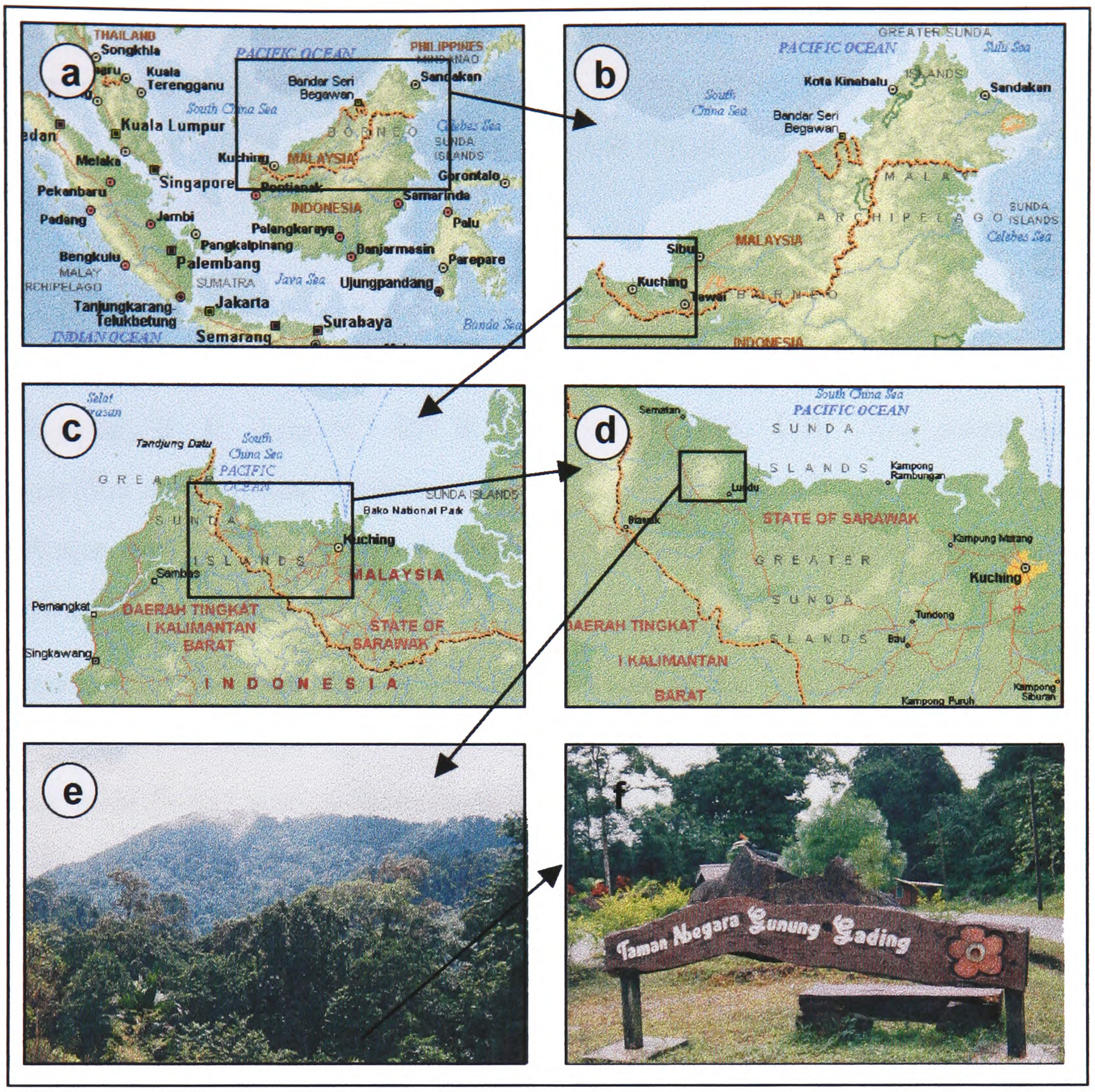


Figure 4.3 The location of the study site. (**a**) Malaysia in South East Asia; (**b**) Sabah and Sarawak, Malaysia; (**c**) South of Sarawak, Kuching and surroundings; (**d**) Lundu area; (**e**) Gunung Gading National Park; (**f**) the forest. (Source of maps: (Expedia.com® 2000)).

4.2 Methods

4.2.1 Plant material

Rafflesia tuan-mudae Becc. is an endemic species from Sarawak, (Malaysia) confined to three isolated mountains Pueh, Gading and Rara. *R. tuan-mudae* usually grows in rich alluvial or limestone-derived soils and is restricted to primary and secondary forest of altitudes below about 2000 m. (Meijer 1997) says the flower has a diameter range between 44 - 56 cm, although (Beccari 1868) reports an individual of 86 cm in diameter and one female flower of the present study measured 78 cm diameter.

4.2.2. Study site

This study was conducted in Taman Negara Gunung Gading, Lundu, Sarawak, Malaysia, S. E. Asia (1° 40' N 109° 52' E) (Fig. 4.3). The forest here is classified as a lowland Dipterocarp forest (50-883 m asl) (Beccari 1902), cited by (Meijer 1997). The climate is aseasonal, with mean monthly rainfall exceeding 100 mm for all months.

Three young buds and five flowers were located in the understory of dense canopy and studied in natural conditions, during two visits in 1998 and one visit in 1999. One male flower and the three buds were investigated from the 14th to the 27th of July, three female flowers from the 27th of September to the 12th of October 1998 and one male flower from the 13th to the 17th of January 1999. Three flower stages were classified as: (1) young bud, three to one month before anthesis, (2) fresh flower, the first and the second days following anthesis and (3) decaying flower, from the third day following anthesis.

4.2.3 Field measurements

The procedures described below were applied to all the flowers in this study unless otherwise specified.

Temperature of buds, flowers and the microclimate

PVC-insulated copper-constantan (0.5 mm in diameter) thermocouples were used to measure the temperatures of buds and floral structures. The thermocouples were calibrated against an Hg thermometer. To place the thermocouples in the interior (column) of the buds (Fig. 4.4a), they were first threaded in 7-20 cm hypodermic

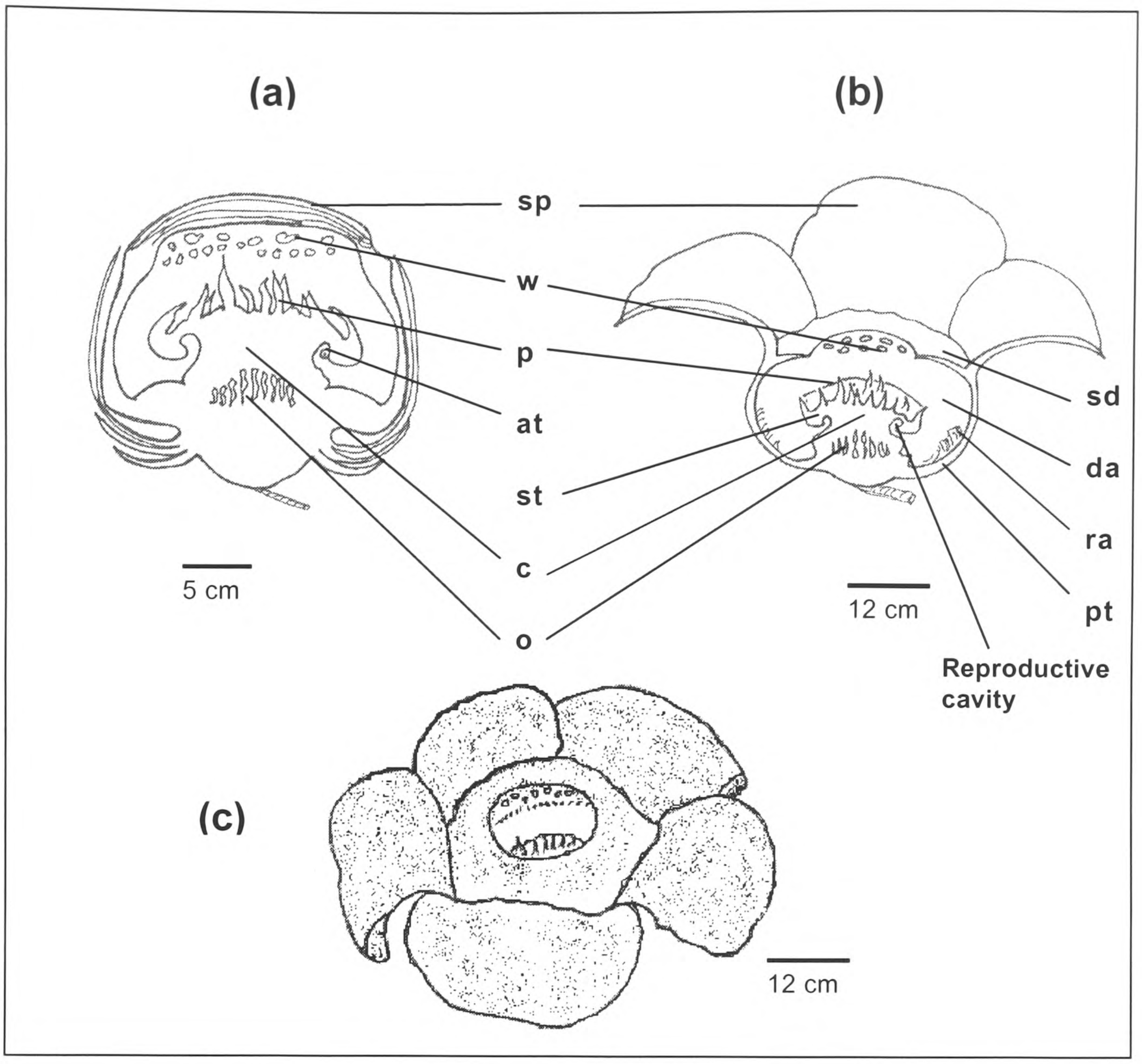


Figure 4.4 A schematic representation of a median longitudinal section through: (a), male bud; (b), female flower; (c), open female flower of *R. tuan-mudae* showing the floral structure. The symbols are: (*sp*) surface of the perigone lobe, (*w*) windows, (*p*) process, (*at*) anther area of the male flowers, (*st*) stigmatic area of the female flower, (*c*) column, (*o*) ovary, (*sd*) surface of the diaphragm, (*da*) space of the diaphragm, (*ra*) ramentae (*pt*) surface of the perigone tube.

needles, which were fully inserted into the centre of the bud and then carefully removed by slipping the wire through the needle leaving the end of the thermocouple at the desired place. The temperature measurements of open flowers started on the first day of blooming. Thermocouples were placed in different parts of the flower (Fig. 4.4) to measure the temperature at: the centre of the disk or column (*c*), anther area of male flowers (*at*), stigma area of female flowers (*st*), process (*p*), surface of the perigone lobe (*sp*), surface of the diaphragm (*sd*), window (*w*) and surface of the perigone tube (*pt*). An additional thermocouple was placed inside the diaphragm without touching the tissue to measure the temperature of the air inside the diaphragm (*da*) (Fig. 4.4a & 4.4b). Soil temperature (T_{so}) was also recorded with a thermocouple 5 mm below the soil surface. Dry-bulb temperature (air temperature, T_a) and wet-bulb temperature were measured with thermocouples shaded in a custom-built forced-air hygrometer placed at 10-30 cm above the ground. Relative humidity and water vapour pressure were calculated from these temperatures (Chapter 2, equation 2.44). Wind velocity, u, at the flower surface was measured with an omnidirectional hot-wire anemometer (model 8460--13E-V, TSI Inc., St. Paul Minnesota). This anemometer was used only during the day and disconnected when rain occurred. A cup anemometer (Model MG2, Vector Instruments) was used permanently during night and day. This anemometer was placed at flower level. The net radiation, R_n, was measured with a Funk type net radiometer (Q*7 Net Radiometer, Campbell Scientific Ltd., Leicestershire, UK). The net radiometer was placed near the flower at the same level. Photosynthetic photon flux density (PPFD) was recorded with a quantum light sensor (Quantum Sensor SKP215, Skye Instruments Ltd. Llandrindod Wells, Powys, Wales, UK) to determine when the flowers were illuminated by sunflecks. The sensor was placed at the same level as the net radiometer.

All variables for the two male flowers and the three buds were measured every 20 s and recorded as means of 15 readings once per 5 min during the night and the day. For the three female flowers the variables were measured every 6 s and recorded as means of 10 readings once per min during the day, and every 20 s and recorded as means of 15 readings once per 5 min during the night on a logger (Model 21X, Campbell Scientific, Ltd., Leicestershire, UK). They were subsequently plotted as average 10-min values.

4.3 Results

Temperature of buds, flowers and microclimate

Diurnal changes in tissue temperature and microclimate variables were studied for 14 days on three young buds of undetermined sex and 5-8 days on two male and three female flowers. The buds available for study did not reach maturity because termites destroyed them at the end of the second week of measurements.

Fig. 4.5a-d shows a representative 9-day course of tissue temperature and microclimatic variables for the three buds. The data represent the average of interior (column) and surface tissue temperatures for the three buds, along with the record of microclimatic conditions within the local area of about 4 m^2. Note that measurements of R_n, PPFD (Fig. 4.5c), and u (Fig. 4.5d) were only measured for 7 days. Air temperature fluctuated from 22.1 to 27.5 °C while the internal temperature fluctuated between 23.4 and 26.0 °C. Internal temperature lagged behind air temperature, peaking in the early evening (Fig. 4.5a). The surface temperature closely followed the pattern of air temperature. During the warmer days (days 2, 4 a nd 9) the interior and surface temperatures were lower than the air with a minimum difference of -2.8 K suggesting evaporative cooling. During days 6–9 there is evidence of metabolic heating, with excess temperatures (0 - 2 K) occurring most of the time (Fig. 4.5b). Here, the term "excess temperature" means the extent to which the tissue is warmer than the air and is expressed in K. In this period, the interior temperatures usually exceed the surface temperatures (Fig. 4.5b). The maximum excess temperatures were remarkably consistent between buds, usually varying by ± 0.04 K. Net radiation was usually less than 15 W m^{-2} and PPFD was usually less than 10 $\mu mol\ m^{-2}\ s^{-1}$, with a maximum of 27 $\mu mol\ m^{-2}\ s^{-1}$ on day 2 as a result of a sunfleck (Fig. 4.5c). Wind velocity at the bud level never exceeded 0.4 m s^{-1} and VPD was usually less than 0.3 kPa during

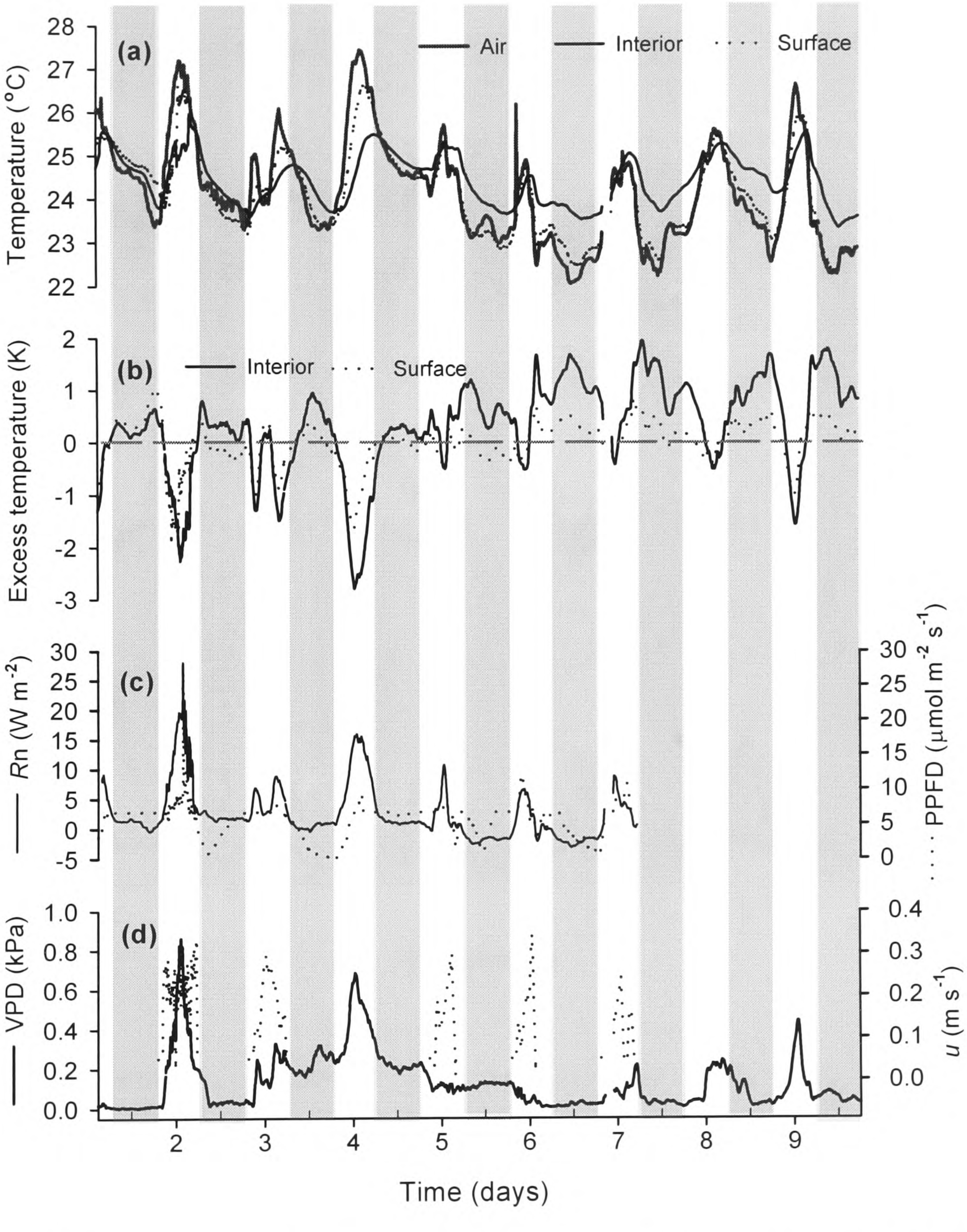


Figure 4.5 Temperature and the microclimate of three buds of *R. tuan-mudae* from the 14th to 24 October 1998. Nights are indicated by grey shadows. (a), temperature of the air (continuoubior (continuous line) and surface (dotted line); (c), net radiation (continuous line) and photosynthetic photon flux density (PPFD) (dotted line); (d), vapour pressure deficit (continuous line) and wind velocity (dotted line).

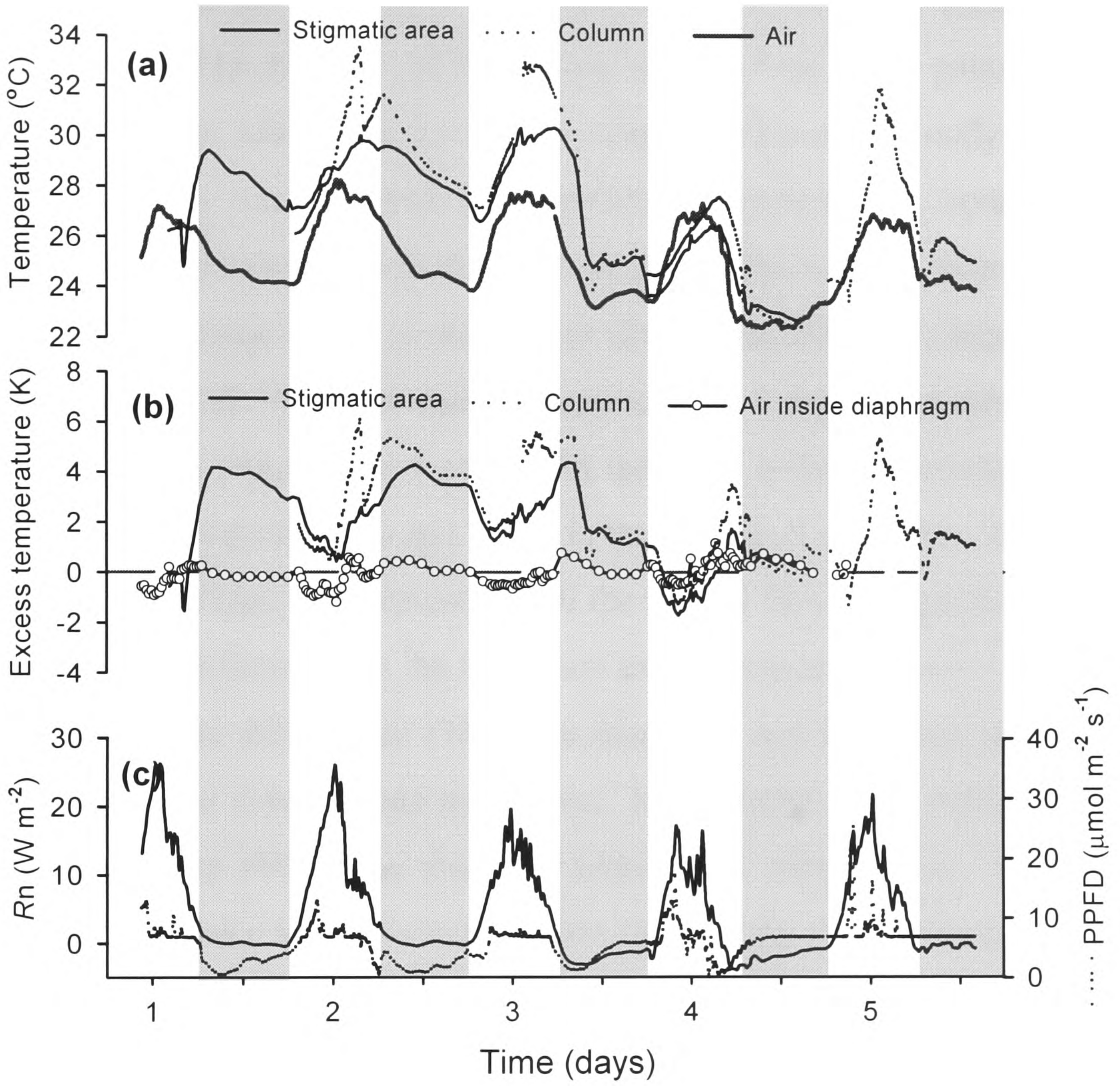


Figure 4.6 Excess tissue temperature and measured net radiation of a female flower of *R. tuan-mudae* during a 6-day period from the 3rd to 9th October 1998, from the day of anthesis until flower decayed. Nights are indicated by grey shadows; (a), lines represent column (dotted line), stigmatic area (continuous thin line), and air (thick continuous line); (b), lines are stigmatic area (continuous line), column (dotted line) and the air inside the diaphragm (continuous line and open circle); (c), measured net radiation (continuous line) and photosynthetic flux density (dotted line).

the whole period so evaporation is unlikely to have contributed substantially to the heat balance of the buds except during warmer days (days 2, 4 and 9) (Fig. 4.5d). The patterns of microclimate were similar for all the five blooming flowers (data not shown). Figure 4.6a-c represents a 6-day course of excess tissue temperature, net radiation and air and tissue temperature for a blooming female flower in October 1998. The diameter of this flower was 49.5 cm. The patterns presented in this figure were similar for the five blooming flowers of this study regardless of the sex, but only one is shown for simplicity of presentation (some differences between individual flowers will be noted below). The internal tissue was usually higher than air temperature, in this flower the column was the warmer part of the reaching almost 34 °C in the early afternoon (Fig. 4.6a) and presents a trend in which the excess temperature increases in the early evening reaching a maximum of 4.2 K in the stigmatic area and 5.8 K in the column (Fig. 4.6b). This pattern in the excess temperature was repeated in all the studied flowers regardless of the sex. The were some differences in the maximum excess between flowers. For example, one bigger female flower size (78 cm in diameter) had an excess temperature of about 2.2 K in the column (data not shown). Net radiation never exceeded 30 W m ^{2}. The maximum PPFD was about 25 μmol m^{-2} s^{-1} (Fig. 4.6c). There was no significant difference in excess temperature between the anther and the column for the male flowers suggesting that the visit of the pollinating flies to female and male flowers is performed in the same way.

The excess temperature of the tissue was sometimes negative (-0.5 to -1.5 K) during the day, usually when it was raining. The air temperature inside the diaphragm of the flowers sometimes exceeded that of the external air, reaching a maximum excess of 1K (Fig. 4.6b).

Figure 4.7 and 4.8 show a schematic representation of tissue temperature for female and male flowers during the day. The day is divided into four 6h periods.

Figure 4.7

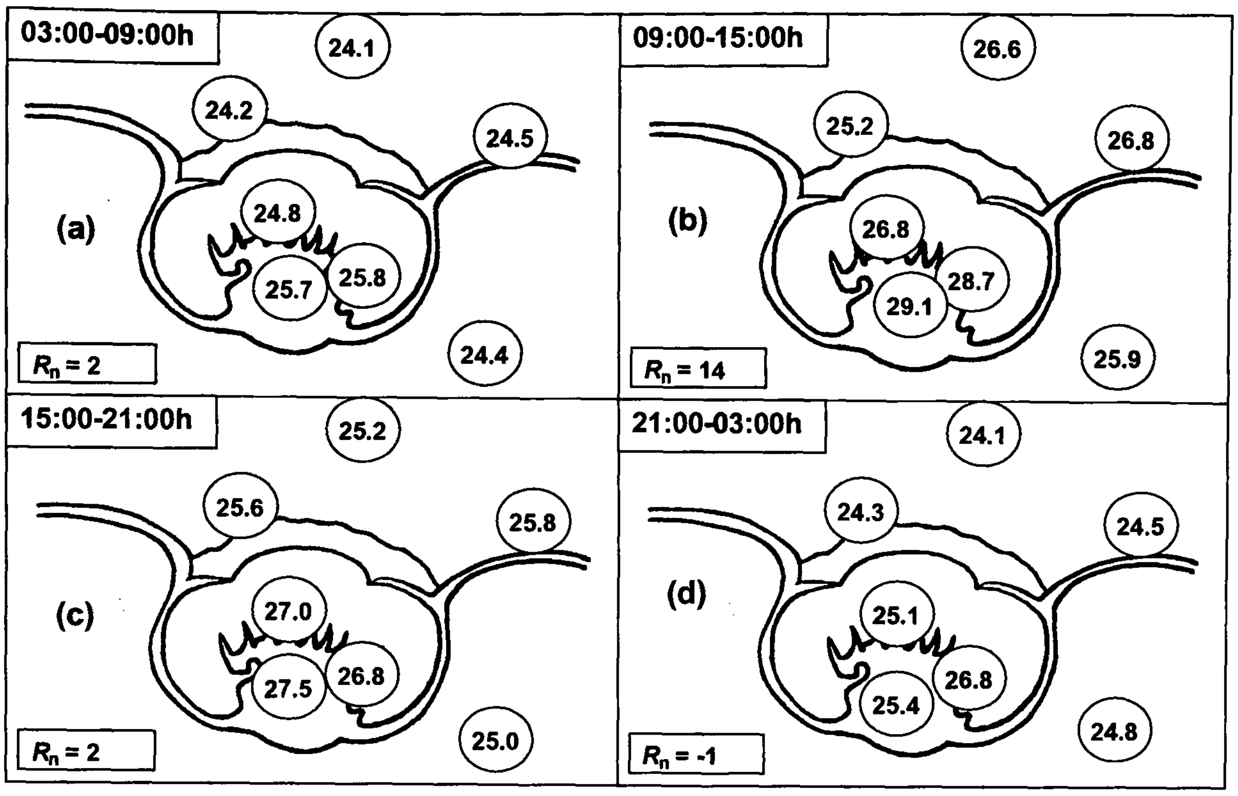
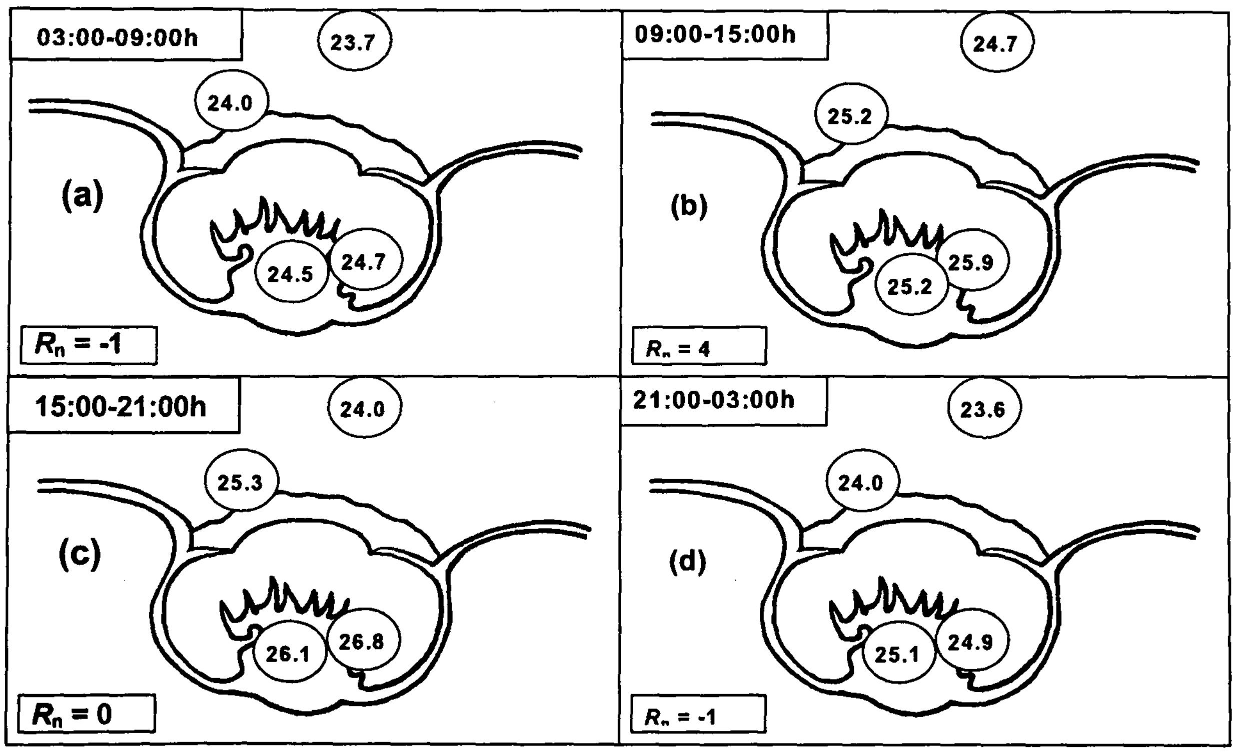


Figure 4. 7 & 4.8 Schematic representation of tissue temperature of a female flower (up) and male flower (down) during the day. The day is divided into four periods of six hours each. Each temperature value represents the period mean temperature of 6-day measurements. (**a**), period from 03:00 to 09:00 h, (**b**), period from 09:00 to 15:00 h (**c**), period from 15:00 to 21:00 h, (**d**), period from 21:00 to 03:00 h. The numbers inside ovals are the mean values of temperature in °C. Net radiation (R_n) is given in W m^{-2}.

Figure 4.8



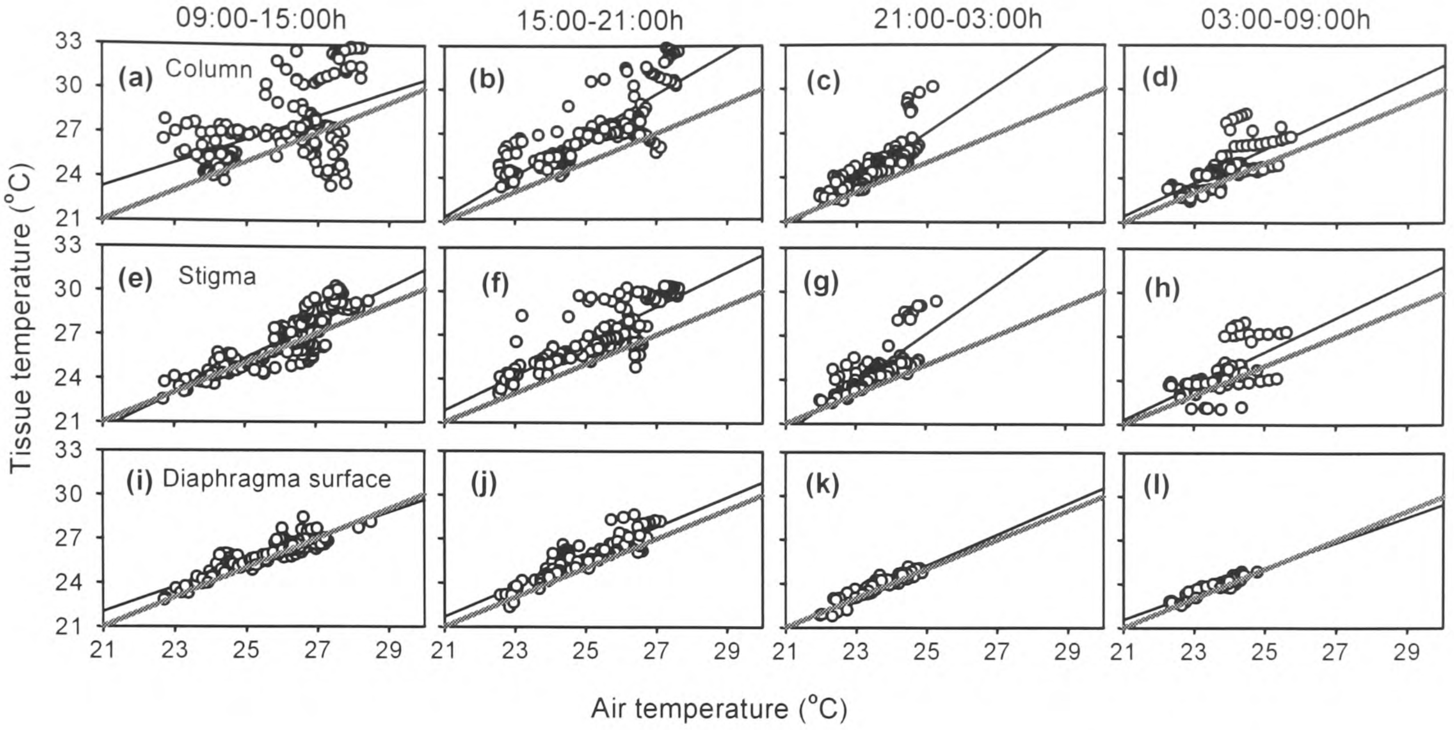


Figure 4.9 Correlation between air and tissue temperature of three female flowers: **(a-d)** column; **(e-h)** stigmatic area; and **(i-l)** diaphragm surface. To simplify the plots data are plotted as one out of every five readings of the original data. The bold line indicates the one-to-one relationship of air temperature... The day is divided into four periods: 09:00-15:00h **(a,e,i)**; 15:00-21:00h **(b,f,j)**; 21:00-03:00h **(c,g,k)**; and 03:00-09:00h **(d,h,l)**. The column was consistently warmer than the air mainly during the day (09:00-15:00h and 15:00-21:00h), the stigmatic are was slightly warmer during the afternoon (15:00-21:00h).

Each temperature represents the mean of 6-day measurements for each period. The measured net radiation (R_n) is also shown. The maximum temperatures were attained in the column and stigmatic area between 09:00 - 15:00, (Fig. 4.7b) with the column reaching 29.1 °C, the stigmatic area reaching 28.7 °C whilst the air temperature was only 26.6 °C and the soil temperature was 25.9 °C. The second warmest period inside the flower was the late afternoon (from 15:00 to 21:00 h, Fig. 4.7c). For all the flowers the column showed the most warming with maximum values normally fluctuating between 27.3 and 33.6 °C (data not shown) while the air temperature fluctuated between 26.1 and 29.1 °C. To characterise the thermal relationships between flower parts and air temperature during the blooming period (fresh flower only), data from 6-hour periods (09:00-15:00h, 15:00-21:00h, 21:00-03:00h, and 03:00-09:00h were plotted as a sequence of scatter plots.

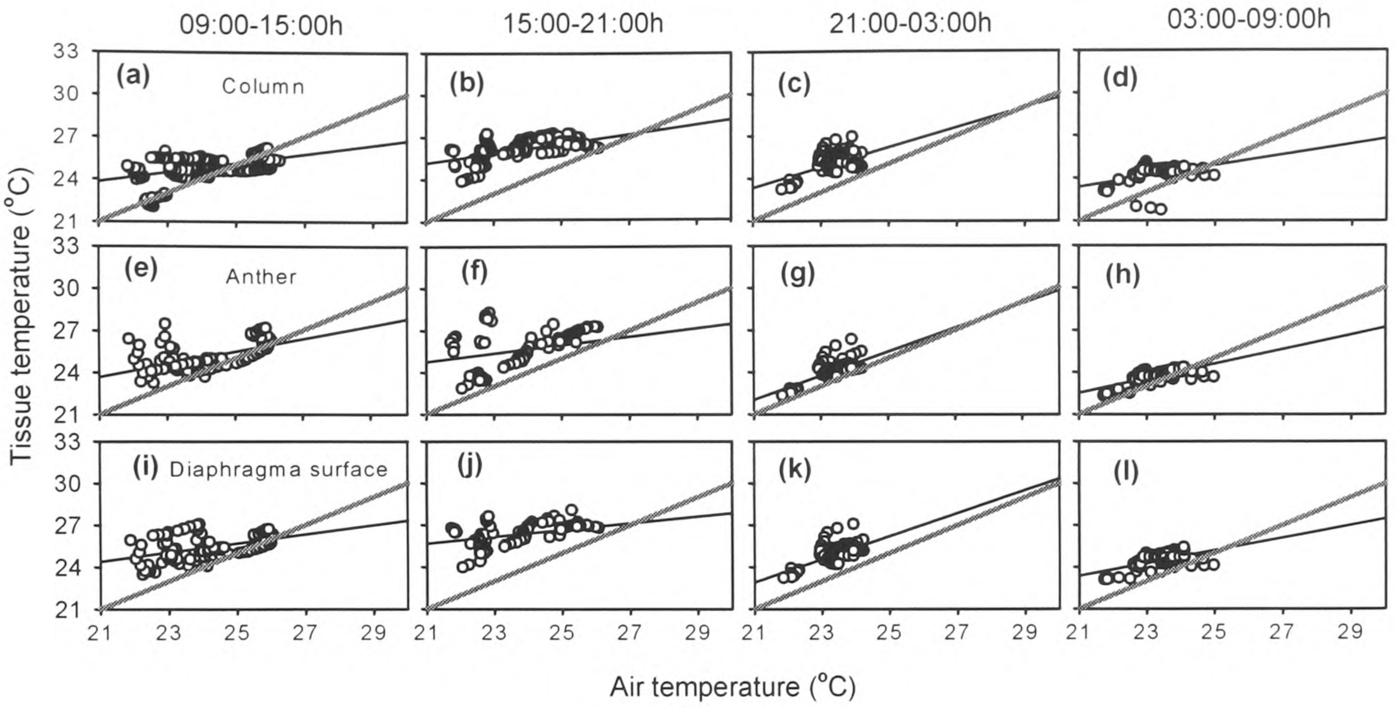


Figure 4.10 Correlation between air and tissue temperature of two male flowers: **(a-d)** column; **(e-h)** anther area; and **(i-l)** diaphragm surface. To simplify the plots data are plotted as one out of every five readings of the original data. The bold line indicates the one-to-one relationship of air temperature. The day is divided into four periods: 09:00-15:00h (**a,e,i**); 15:00-21:00h (**b,f,j**); 21:00-03:00h (**c,g,k**); and 03:00-09:00h (**d,h,l**). All parts were consistently warmer than the air mainly during the day. There is some evidence of endothermy, with tissue temperature exceeding air temperature when air temperature was low.

Figure 4.9 shows the sequence of plots for three female flowers and Figure 4.10 for two male flowers in relation to the 1:1 relationship between air temperature (shown as a bold line) and tissue temperature. Internal tissue temperatures tended to be higher than the air during the day and early evening regardless of the sex. (Figs. 4.9a,b,e,f and 4.10a,b,e,f). The column was the warmer part, predominantly during the day and the early morning (Figs. 4.9a,b and 4.10a,b) while the anther area of male flowers (Fig. 4.9e,f) seems to be warmer than the stigmatic area of female flowers (Fig. 4.10e,f). During the day and in the early evening tissue the temperature of the male flowers (Fig. 4.9a,b,e,f,i,j) tended to be more constant than the females flowers (Fig. 4.10a,b,e,f,i,j) this is shown by the slope of the regression lines tending to be one intersecting the 1:1 line.

4.4 Discussion

In this study it is concluded that *R. tuan-mudae* is an endothermic flower, showing a maximum of 6 K of excess tissue temperature in one flower, but not as endothermic as the related species *Rhizanthes lowii* (Patiño *et al* 2000) and other well known thermogenic, beetle pollinated flowers belonging to the families *Araceae*, *Aristolochiaceae* (Knoll 1926; Meeuse 1966; Knutson 1972; Meeuse

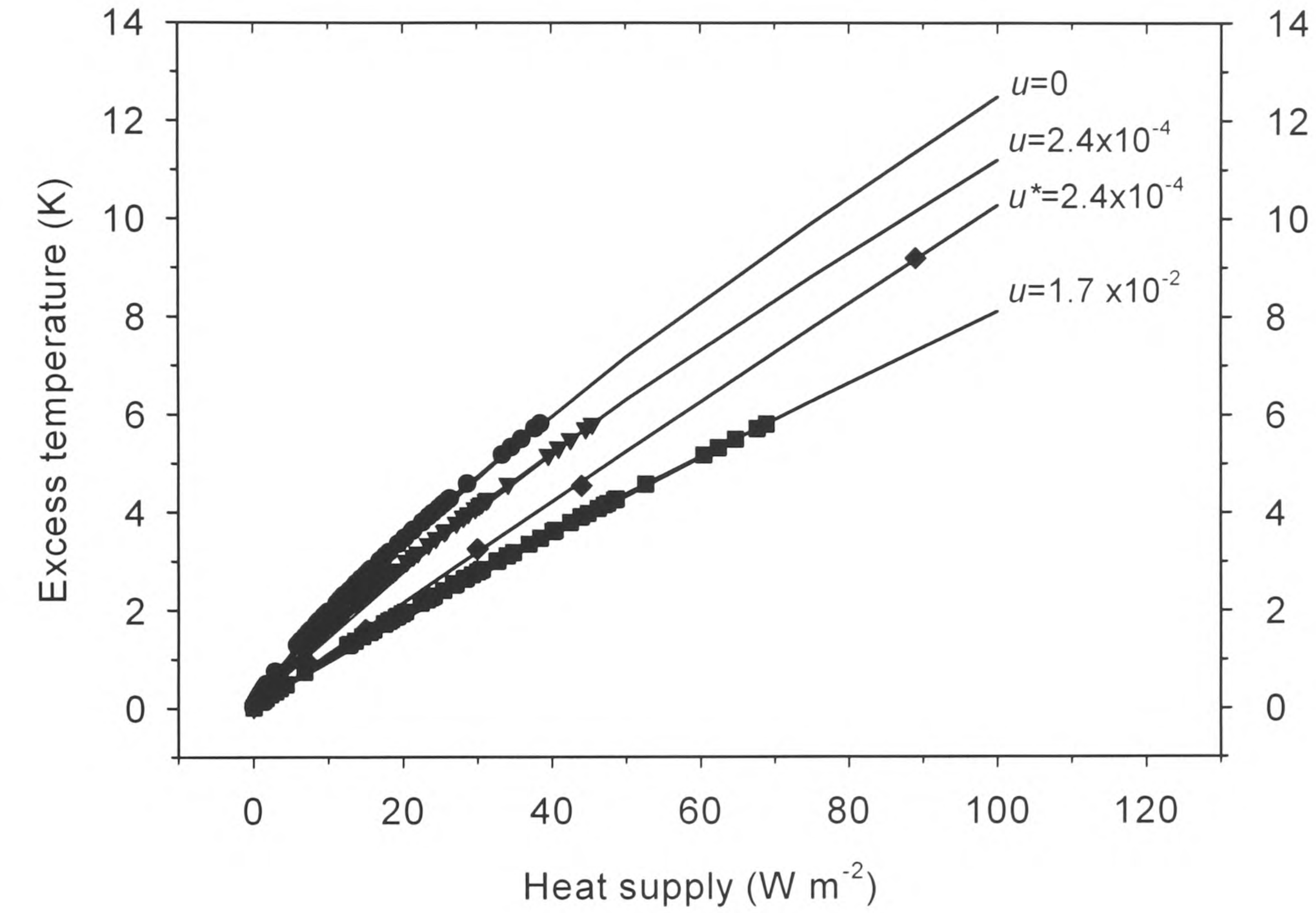


Figure 4.11 Calculation of the heat supply needed to rise the temperature of the flowers to the range observed for different wind speeds (u, m s^{-1}) inside the diaphragm. The symbols indicate the calculated heat supply made using method 1 by using the observed temperature of the flower surface in the understory of the forest. The calculated heat supply by method 2 using FLUENT software is represented by the diamond and u*. The air flow inside the diaphragm was calculated with FLUENT. An air flow of 2.4 x10-4 m s^{-1} inside the diaphragm corresponds to a wind speed of 0.2 m s^{-1} in the understory and an air flow of 1.7 m s^{-2} corresponds to a wind speed of 1 m s^{-1} in the understory.

1975; Meeuse and Raskin 1988; Uemura et al 1993), *Annonaceae* (Gottsberger 1989; Gottsberger 1990), *Arecaceae, Cyclanthaceae* (Gottsberger 1990) *Cycadaceae* (Skubatz et al 1993), *Nymphaeaceae* (Prance and Arias 1975), *Magnoliaceae* (Dieringer et al 1999), *Nelumbulacea* (Miyake 1898; Schneider and Buchanan 1980; Seymour and Schultze-Motel 1996).

Endothermy in *Rafflesia* has been suggested previously (Meeuse 1978; Beaman et al 1988) and efforts to detect heating were made (Nais 1997). Nais's studies on *R. keithii* Meijer in Sabah did not reveal any significant excess tissue temperature. There were important differences in the methodology of the two studies. In the present study the thermocouples were inserted inside the tissue and recorded the temperature every 1-5 min during the whole period of blooming (usually 7 days). 1.5 - 6.0 K of excess temperatures were detected in the internal parts of the flower. In Nais's study, only the surface temperatures were measured, as spot readings, with a probe, which was held against the surface.

During the day the buds were cooler than the air (Fig. 4.3b), suggesting that evaporative heat transfer may be significant, and may affect the thermal balance of the buds, cooling down the surface when the radiation load from the surroundings is higher and VPD increases. In the present study it was not possible to measure the stomatal conductance or transpiration rate of the floral surface, but stomata are present in the adaxial surface of the perigone lobe (Cammerloher 1920). Consequently it was not possible to construct the complete energy budget of the flower.

The possibility that the energy for heating the tissue by up to 6 K is not derived from metabolism but from radiation was explored (Fig. 4.11). To do this, the heat supply required to raise the tissue temperature of the central column of a female flower several degrees above the ambient was calculated using two different approaches. 1) The method described by Van Gardingen & Grace (1991). In this method, the heat transfer is modelled as a combination of forced and free

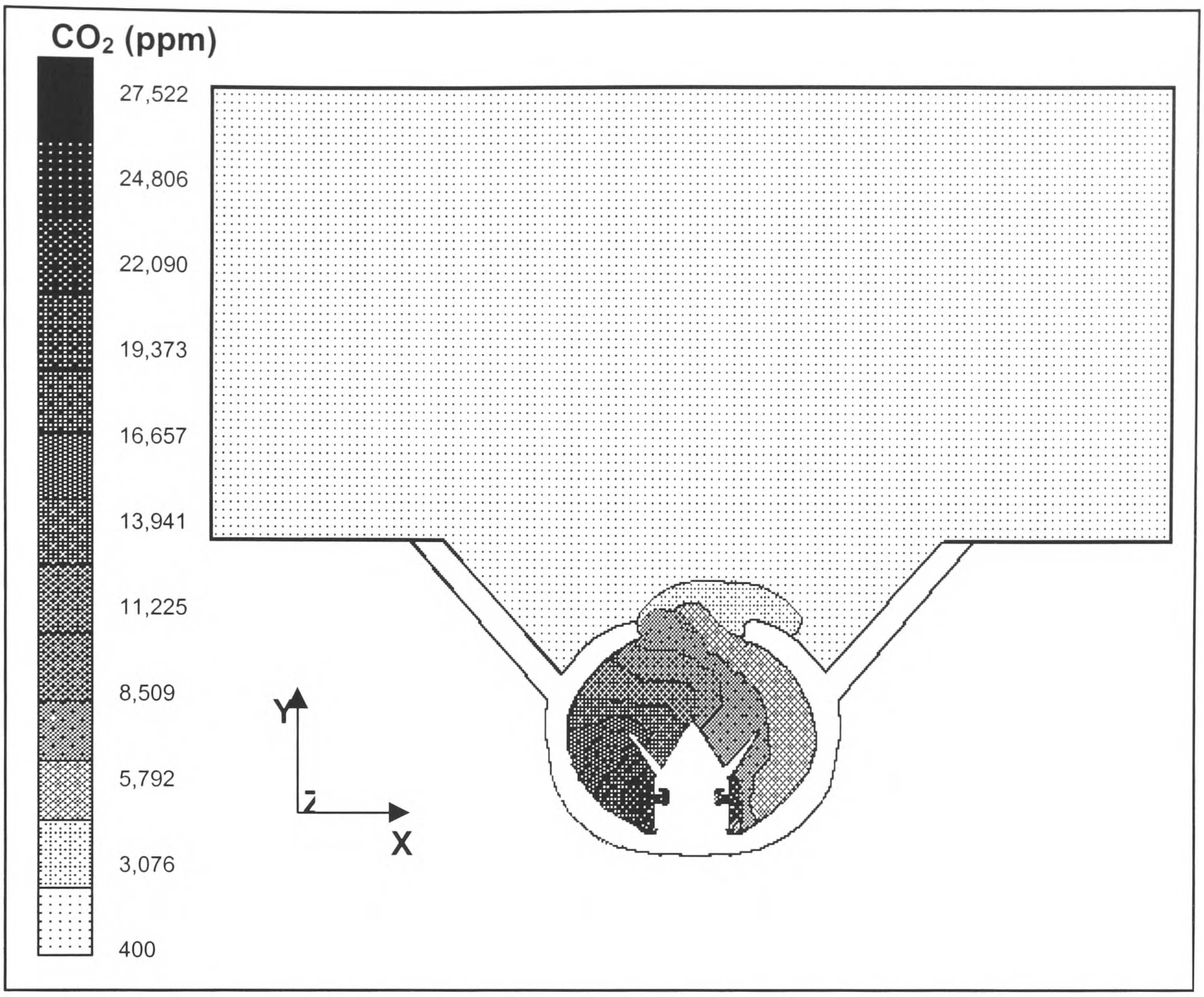


Figure 4.14 Map of CO_2 concentration inside the flower. The CO_2 concentration is estimated by FLUENT, on the basis of the observed rise in temperature. Respiratory activity was assumed to be confined to the central column where the cavity with the reproductive parts is located.

convection (Chapter 2). As no data for the stomatal conductance of the central column is available, we assumed that the surface was completely dry. 2) An exact method using a computational fluid dynamics model (in collaboration with Tuula Aalto and Timo Vesala, Physics Department, University of Helsinki). Heat and CO_2 transfer in this method is solved using the commercial software package, FLUENT. The solution was based on a finite difference scheme in an adaptive grid and the transfer equations were solved in a segregated mode. Flow past the flower was set to the measured value of 0.2 m s^{-1} and it was assumed to be turbulent. Free convection due to temperature differences was also included, since the wind velocities near the central column were low. Physical properties of the flower material (conductivity, specific heat capacity) were assumed to be the same as water.

The CO_2 production rate was solved from heat production given knowledge of the heat of combustion of glucose, and assuming 6 CO_2 molecules are produced per one glucose oxidised. According to both the models the heat supply necessary to produce an excess temperature of 3 - 6 K in a dry flower at an air flow of 2.4×10^{-4} m s^{-1} inside the diaphragm would be about 50 - 60 W m^{-2}. Of course, given some evaporative cooling, the heat supply would need to be more than this. As the net radiation measured over the flower was much less than 50 – 60 W m^{-2} (usually less than 15 W m^{-2}), it was conclude that metabolic heat must have been an important part of the heat supply.

Further evidence that radiation is not the most important component of the heat supply comes from comparison of the time courses of the flux of radiant energy and (calculated) convection. When net radiation was maximal, convective heat loss was at a minimum (Fig. 4.12), suggesting that the energy is produced internally and is not from the net radiation in the forest understory.

The concentration of CO_2 obtained with FLUENT in the stigmatic area is about 4-17 times higher than near the surface of the diaphragm, 76 times higher than at the surface of the flower and approximately 76 times compared with the understory concentration (Fig. 4.13). The CO_2 concentration in the understory (1m above the ground) in the Amazonian rain forest at the Reserva Jaru, Brazil varied from 360 in the evening to 570 ppm early in the morning (Kruijt *et al.* 1996)

The question of why endothermy has evolved in a warm environment is an interesting one. It has been observed that pollination is by large blowflies which mistake *Rafflesia* for rotting flesh, festering sores, carrion or animal faeces (Bänziger 1991).

There exists the possibility that high temperatures are a component of the pollination syndrome, as they assist volatilisation of organic compounds such as dimethyl disulphide, dimethyl trisulfide and (1,4), (1,2) and 1,3-diethyl benzene which have been extracted and identified from the interior of the diaphragm of *Rafflesia tuan-mudae* (Patino *et al.* paper in preparation) that are involved in the long distance attraction of the pollinating flies. It is not possible to rule out the possibility that CO_2 is the attracting gas, as some blowflies respond to it. Experiments elsewhere showed that when dimethyl disulphide was mixed with carbon dioxide and applied to sheep it was an attractant of the blowfly *Lucilia sericata* (Cragg and Thurston 1949).

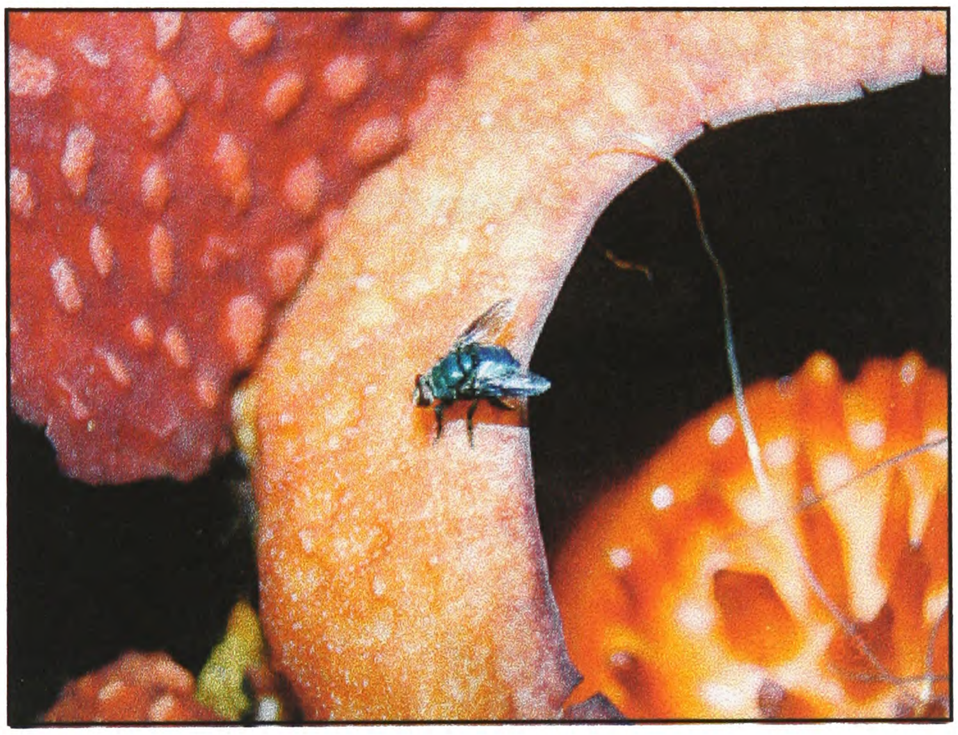


Figure 4.13 A blowfly walking around the central opening of the diaphragm of a female flower.

Dimethyl disulphide together with carbon dioxide plays a role in the attraction of blowflies to sheep, and these gases are released during the breakdown of fleece keratin of sheep, in fact the result of bacterial decomposition of cystine (Cragg and Ramage 1945). Blowflies are active by day, when the CO_2 that has accumulated inside the diaphragm during the stable conditions at night and will be dispersed as a buoyant plume. Dispersal may also be trigged by high temperatures inside the diaphragm (Fig 3b) carrying the volatile chemical signals involved in the attraction of pollinators from long distances. The size and colour of the flower have been suggested to act as visual stimuli in the attraction of the pollinators {Bänziger 1991 ID: 439}. Once the flies are on the flower, usually they walk on the diaphragm surface (Fig. 13) and reach its central opening, then they go inside (Patiño, personal observation), probably attracted by the mixture of volatiles and the high concentration of carbon dioxide relative to the external air that reaches the surface of the aperture (Fig. 14). The flies may be attracted to the reproductive cavity where the maximum carbon dioxide concentrations occur according to the model (Fig. 14).

The presence of carbon dioxide may play an important role in the pollination syndrome of *Rhizanthes lowii* and *Rafflesia tuan-mudae* and perhaps as well in other endothermic flowers as first suggested by (Moodie 1976). Further studies are necessary to test this hypothesis.

4.5 Summary

1. Because *Rafflesia* flowers present several characteristics in common with its relative *Rhizanthes lowii* and other endothermic flowers, the possible existence of endothermy in the giant flowers of *Rafflesia tuan-mudae* (Rafflesiaceae) was investigated.

2. The internal and surface temperature was continuously monitored with fine thermocouples in different parts of the flowers whilst radiation fluxes and microclimatic variables were recorded. The measurements were made over several days, starting on the day of blooming until the flower decayed.

3. It was found that the internal parts of the flower were maintained a few degrees (1-6 K) above air temperature. The maximum heating was in the evening.

4. It is not possible to account for this temperature rise without postulating a significant internal source of heat. It was concluded that Rafflesia tuan-mudae is an endothermic flower that generates a maximum of 50-60 W m-2 of heat in the centre of the column.

5. A computational fluid dynamics model was used by Dr. Tuula Aalto from the Physics Department of the University of Helsinki to predict the concentrations of carbon dioxide inside the diaphragm of the flower. The model suggested that the maximum concentration (around 30,000 ppm) of carbon dioxide was developed mainly around the central column where the cavity with the reproductive parts (anther or stigma) is located.

6. The possible role of endothermy, carbon dioxide and volatiles as elements in the mimicry of rotten flesh or infected sore as attractants to pollinating blowflies is discussed.

Chapter 5

The cooling of convolvulaceous flowers in a tropical environment

5.1 Introduction

In attracting insects, flowers are necessarily exposed to full solar radiation. In a cold climate this exposure is advantageous, as the floral parts are warmed several degrees above air temperature. In such cases, the high temperatures may serve as a reward for pollinating insects (Cooley 1995; Hocking 1968; Hocking and Sharplin 1965; Kudo 1995; Smith and Meeuse 1966) and may enhance the rate of floral development (Kevan 1975; Kjellberg, Karlsson and Kerstensson 1982; Kudo 1995; Totland 1996). Such flowers seem to be structurally adapted to maximise the warming effect. For example some flowers present the appropriate paraboloid

Figure 5.1 Convolvulaceous flowers growing in their natural habitats close to the ground; (left) *Merremia borneensis*; (right) *Ipomoea pes-caprae*.

shape to capture solar radiation (Hocking and Sharplin 1965; Kevan 1975), other flowers are heliotropic (Hocking 1968; Kevan 1975; Kjellberg et al. 1982; Krannitz 1996; Kudo 1995; Stanton and Galen 1989; Totland 1996) while some have pubescent inflorescences to minimise convective heat loss (Miller 1986) and others have spectral reflectances that ensure that the gynoecium is warmer than the air (Jewell, McKee and Richards 1994).

Work on floral temperature has usually focused on flowers in alpine, subarctic and temperate climates where an increase of floral temperature has a positive effect on the development of the flower (Kevan 1975; Krannitz 1996; Kudo 1995; Stanton and Galen 1989; Totland 1999). However, in hot climates this exposure to solar radiation, and the elevated temperatures of the floral parts, may be damaging. This may be especially true for plants with flowers close to the ground (Fig. 5.1), where mid-day temperatures can exceed 40 °C (personal observations). In such conditions, transpiration cooling, and other mechanisms may be crucial to survival, just as it is sometimes in leaves (Althawadi and Grace 1986).

In this chapter I present the first study of the floral temperature and energy balance of ground-dwelling flowers in a tropical environment.

5.2 Methods

5.2.1 Plant material

Experiments were made on three species belonging to the family Convolvulaceae Juss. Members of the Convolvulaceae are mostly twining herbs or shrubs, comprising about 85 genera and 2,800 species. The flowers are radially symmetrical and nearly always bisexual. They resemble a funnel and are heliotropic (Fig. 5.2). The perianth and androecial whorls are 5-merous. The petals are fused into a funnel-shaped corolla, almost unlobed. The sepals of the calyx are usually distinct. The stamens are inserted near the base of the corolla tube, or midway down the corolla tube and are often unequal and alternate with the

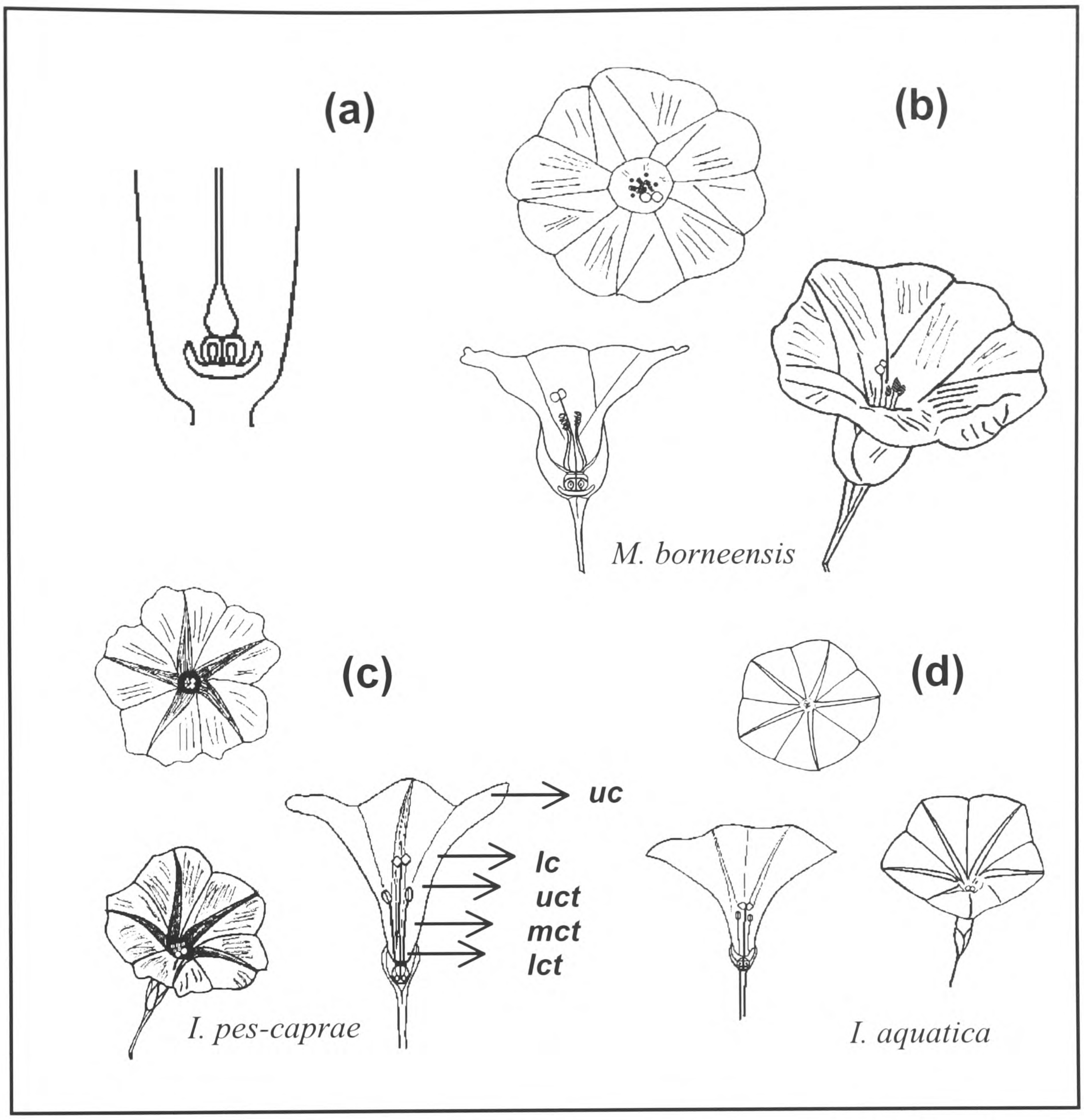


Figure 5.2 A schematic representation of the three convolvulous species showing (a) general arrangement of the ovary, cross-section; horizontal view, median longitudinal section and whole flower of: (b) *Merremia borneensis*; (c) *Ipomoea pescaprae*; (d) *Ipomoea aquatica*. The arrows indicate the location of the stomata: *uc*=upper corolla, *lc*=lower corolla, *uct*=upper corolla tube, *mct*=middle corolla tube, and *lct*=lower corolla tube..

lobes. The gynoecium consists of a single compound pistil of two carpels and a superior ovary of two or sometimes up to five locules, each with one or two axile ovules (Fig. 5.2a). The flowers are short lived lasting less than one day. The corollas open in the morning (hence the common name, Morning Glory), wilt around noon and fall early in the afternoon, then the sepals close, assuming the shape of the developing bud (Van Steenis 1953).

Three species are common in Brunei Darussalam, Borneo: *Merremia borneensis* Merr. (Fig. 5.2b), *Ipomoea pes-caprae* (L.) Sweet *ssp brasilensis* (L.) Oostr. (Fig. 5.2c), and *Ipomoea aquatica* Forsk (Fig. 5.2d).

Table 5.1 Summary of the morphological characteristics of the flowers. The flowers of *M. borneensis* last 2-3 h longer than the flowers of *I. pes-caprae* and *I. aquatica*. *D*= diameter

Species	Colour	Gynoecia *D* (mm)	Corolla *D* (mm)
I. pes-caprae	Purple	5.5 ± 0.5	65 ± 5
I. aquatica	White	4.8 ± 0.4	55 ± 5
M. borneensis	Yellow	18.3 ± 0.6	95 ± 10

5.2.2 Study Site

Flowers of *I. pes-caprae* were studied in natural conditions in an open sandy area located at the Universiti Brunei Darussalam campus (4° 58.6' N 114° 53.8' E, elevation 2 m), and on the beach in Pantai Meragang Brunei (Fig. 5.3). Several plants of *I. aquatica* were collected from a drain along Jalan Bebatik Kilanas, Bandar Seri Begawan, and planted in a flooded area at the University campus. Flowers of *M. borneensis* were studied in two open areas in the University campus.

5.2.3 Field measurements

Temperature and microclimate

Flower temperature and microclimate were recorded on flowers growing naturally in the field during April, November and December 1997, August, September and December 1998 and January 1999. Microclimatic variables were measured to enable solution of the energy balance equation. Temperatures were measured using copper-constantan thermocouples (0.5 mm in diameter), which were calibrated against an Hg thermometer. The thermocouples were inserted in the centre of the gynoecium (T_{gy}) and in the corolla (T_{co}) of each flower. The thermocouples were inserted in the corollas from the upper to the lower surface and fixed to the flower surface using the glue from sellotape®. After the corollas were excised the

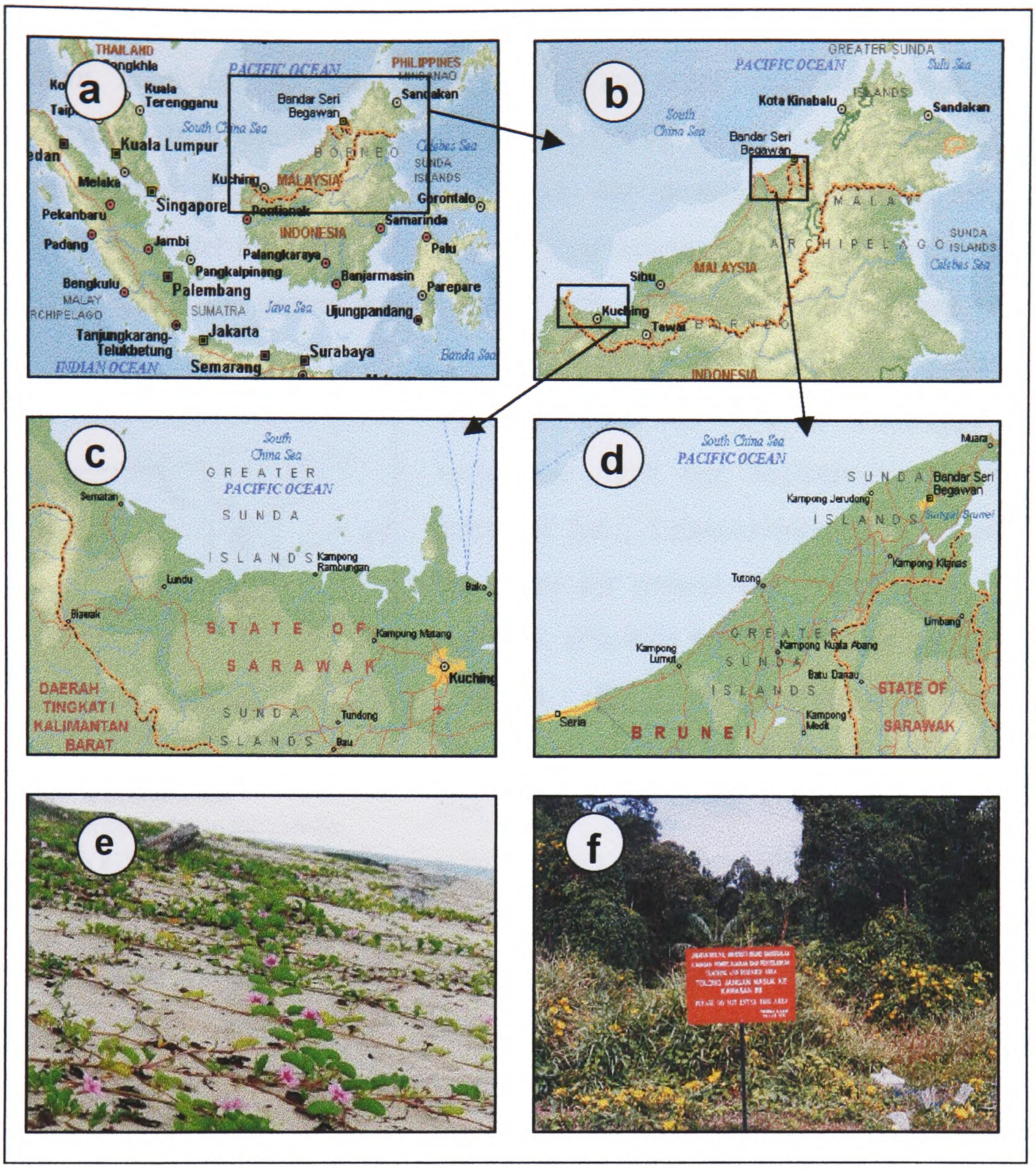


Figure 5.3 Maps showing the study sites along the coast of Brunei and Sarawak. **(a)** Location of Borneo Island in South East Asia; **(b)** the west coast of Borneo; **(c)** the coast in Sarawak; **(d)** the coast of Brunei; **(e)** Pantai Meraggang, Brunei; and **(f)** University of Brunei research area. (Source of maps: (Expedia.com® 2000)).

thermocouples were re-located on the surface of the sepals (T_{se}). Soil temperature (T_{so}) was also recorded with an additional thermocouple covered with a thin layer of soil to avoid exposure to direct sunlight. Dry-bulb temperature (air temperature, T_a) and wet-bulb temperature were measured with thermocouples shaded in a custom-built, forced-air hygrometer placed at 10-30 cm above the ground. Relative humidity and water vapour pressure were calculated from these temperatures (Chapter 2, equation 2.44). Wind velocity (u) at the level of the flower surface, 10-15 cm from the ground, was measured with an air velocity transducer (Model 8475-150, TSI Inc., St. Paul Minnesota, USA). Wind velocity at 50 cm above the ground was measured with a cup anemometer (Model MG2, Vector Instruments, Rhyll, UK). Net radiation, R_n, was measured with a Funk-type net radiometer (Q*7 Net Radiometer, Campbell Scientific Ltd., Leicestershire, UK) placed 30 cm above the ground near the flowers. Net radiation absorbed by the corollas ($R_{n,co}$) and gynoecia ($R_{n,gy}$) were estimated from a knowledge of their reflectance and surface temperature. The energy balance was calculated according to previous work (Althawadi and Grace 1986; Dixon and Grace 1983; Grace 1983; Grace, Fasehun and Dixon 1980; Hippa, Koponen and Osmonen 1981; Patiño, Herre and Tyree 1994; Van Gardingen and Grace 1991). Photosynthetically active photon flux density (PPFD) was recorded with a quantum sensor (SKP215, Skye Instruments Ltd. Llandrindod Wells, Powys, Wales, UK). The flowers selected for the experiments were all completely exposed to the sunlight, not obscured by other plant parts. Variables were measured every 6 s and recorded as means of 10 readings once per minute on a logger (Model 21X, Campbell Scientific, Ltd., Leicestershire, UK.). They were subsequently plotted as average 10-minute values.

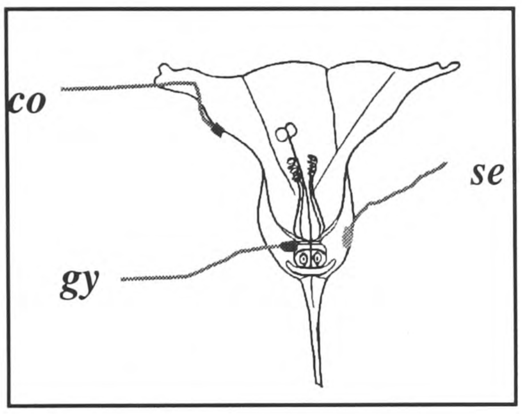


Figure 5.4 Schematic representation of a flower with the location of the thermocouples in the corolla (*co*), gynoecium (*gy*) and sepal (*se*).

In each experimental run, lasting 6-10 hours, temperature of the gynoecium, corolla and sepals of 4-10 flowers were recorded on a data logger. To assess the cooling effect of transpiration, the flowers the sepals and the corollas of half of the flowers were covered with silicone high vacuum grease (Dow Corning Corp., Midland, Michigan) to prevent transpiration, whilst some flowers were used as freely-transpiring controls. The corollas of *I. pes-caprae* were not completely covered with grease because they were extremely fragile (about 1/3 of the corolla was covered). Note that the gynoecium of these flowers is enclosed within the sepals, so the effect of transpiring and non-transpiring sepals will be noticed in the gynoecia temperature. The flowers are short lived. After the corolla folded naturally between 12:00 and 13:00, it was excised. Excised corollas were carefully unfolded to estimate the area of the corolla when the flower is viewed axially ($A_{H,co}$). Then the corollas were flattened on paper to estimate total surface area of the corolla ($A_{T,co}$). The paper area was measured with a leaf area meter (LI-3100, Li-Cor Inc.).

5.2.4 Laboratory experiments

Stomatal density

Several flowers of *M. borneensis I. pes-caprae* and *I. aquatica* were harvested in the morning to estimate stomatal density and size. Thin transverse sections of the surface tissue from corollas and sepals were made with a razor blade and examined with the light microscope. Stomata, when present were counted and measured. A second method to determine the presence of stomata in corollas and sepals was to coat the surface with silicon rubber (Extrude™ wash, Kerr Manufacturing Company, Romulus, MI 48174, USA). After this silicon material had set, the impression was removed from the surface and the resulting replica of the surface was covered with a thin layer of transparent nail varnish. After drying the varnish was peeled away and examined for stomata under the light microscope. The method of calculating $R_{n_{co}}$ is described elsewhere (Patiño et al. 1994).

Evaporative flux density, E_G

Evaporative flux density was measured gravimetrically, using excised flowers of *I. pes-caprae*, outside the laboratory at the Biology Department at the University of Brunei Darussalam, during the same days that temperature measurements were conducted, in November 1997 and August-September 1998. Flowers were brought to the laboratory from the study area, the cut surfaces of the flower stalk and the sepals were greased with high vacuum silicone grease (Dow Corning Corporation, Midland, Mich, USA). Eight corollas were suspended by nylon fishing line over a grass-covered area outside the building in full sunshine from 9:00 to 13:00 and weighed every 30 minutes for a period of 4 h. E_G was calculated from

$$E_G = \Delta W/(\Delta t A)$$

where ΔW is the mass change in time interval Δt and A is the corolla area exposed to sunshine during the measurement period. The $A_{H,co}$ and $A_{T,co}$ were determined as described above.

Temperature treatments

To determine the effect of temperature on flower tissue and pollen viability, flowers of *I. pes-caprae* and *M. borneensis* were exposed to different temperature treatments.

96-100 flowers were collected from the field around 10 am (*I. pes-caprae*) and 11 am (*M. borneensis*), and brought back to the laboratory. The time of collection was chosen because it was assumed that by that time of day natural pollination had occurred.

Once in the laboratory the corollas were removed from the flowers. Four flowers of *I. pes-caprae* were placed inside 2.5 cm x 5 cm glass bottles containing 1 cm^3 of distilled water and four flowers of *M. borneensis* were placed in 6.0 cm x 6.2 cm glass bottles. One thermocouple was inserted in the gynoecium of one of the flowers in each bottle to maintain regular surveillance on the set temperature. Half of the total number of anthers (as a source of pollen) from the four flowers were removed and placed into a vial containing 1 cm^3 of 2.5 % sucrose solution. Six bottles containing flowers and six vials containing pollen were placed in a constant temperature bath. The experimental temperatures were 15, 32, 37, 42 47 and 52 °C. Six additional bottles containing flowers and six containing pollen were keep outside the laboratory at ambient temperature 30 ± 1 °C as a control. After the flowers in the constant temperature bath reached the temperature (set temperature) of the water (time 0), every 60 min, one bottle containing flowers and one bottle containing pollen were taken out of the bath. The bottles taken from the bath were placed together with the control and left overnight to allow the pollen tube to grow.

Next day each flower was dissected; the remaining anthers together with the carpel, and a piece of sepal (0.5 x 0.5 mm aprox.) were placed in a vial containing 1.5 cm^3 (for *I. pes-caprae*) and 5 cm^3 (for *M. borneensis*) of Tetrazolium solution, 0.5 % in phosphate buffer PH 7.5 (TTC), and left again overnight in a dark room at 16 °C (Towill and Mazur 1975). After 24 h the TTC was removed from the bottles and the samples were washed with distilled water. The carpels were transferred into vials containing 3 cm^3 of 95 % Ethanol (to extract the TTC) and the piece of sepal was put in a vial containing 1.5 cm^3 of 95 % Ethanol. After 12-18 h, the carpels, and the pieces of sepals were dried in the oven, and the dry mass was recorded. The alcohol containing the TTC was stored for further spectrophotometer measurements. The remaining pollen was left in 3 cm^3 distilled water and homogenised. 10 mm^3 of this water-pollen solution was extracted and placed in a microscope slide to be counted to give an estimate of pollen viability. The TCC stains viable tissue red. The pollen grains were counted as red stained (live) and non-stained or white (dead).

5.3 Results

Stomatal density

Stomata were present on the abaxial surface of the corollas of *I. aquatica*, *I. pes-caprae* and *M. borneensis* and were concentrated in the middle of the corolla tube (*mct* in Fig. 5.2c) with a few individual stomata in the lower part and none in the upper corolla (Fig. 5.2c). The adaxial surface of the corollas were astomatous. Stomata were numerous on sepals of the same species but their density was lower than in leaves of the same species (Table 5.2).

Table 5.2 Summary of stomatal frequency (mm^{-2}) and pore size (μm) comparing different parts of the plant (corollas, leaves and sepals) for *I. pes-caprae*, *I. aquatica* and *M. borneensis*. The frequency specified for the corolla tube takes into account only the region where the stomata were grouped. For leaves and sepals only the abaxial surface was analysed, for corollas stomata were absent on the adaxial surface.

Species	**Corolla tube**		**Sepal**		**Leaf**	
	Density (s mm^{-2})	Size (μm)	Density (s mm^{-2})	Size (μm)	Density (s mm^{-2})	Size (μm)
I. pes-caprae	1.8		181.6	35	240	19
I. aquatica	16.9	24	79.7	20		
M. borneensis	5.0	15	67.9	28	550	18

Flower temperature and microclimate

The diurnal course of flower temperature and microclimatic variables for *I pes-caprae* over a representative day, the 11th of November 1997, is shown in Figure 5.5. Several experimental runs like this were performed for each species: six for *I. pes-caprae*, five for *M. borneensis* and three for *I. aquatica*. However, only one set of data is presented fully, for simplicity. The maximum net radiation (R_n) approached 700 W m^{-2} around noon and the maximum PPFD was about 1900 μmol m^{-2} s^{-1} (Fig. 5.5 a). Relative humidity was fairly constant (average 55 %) during

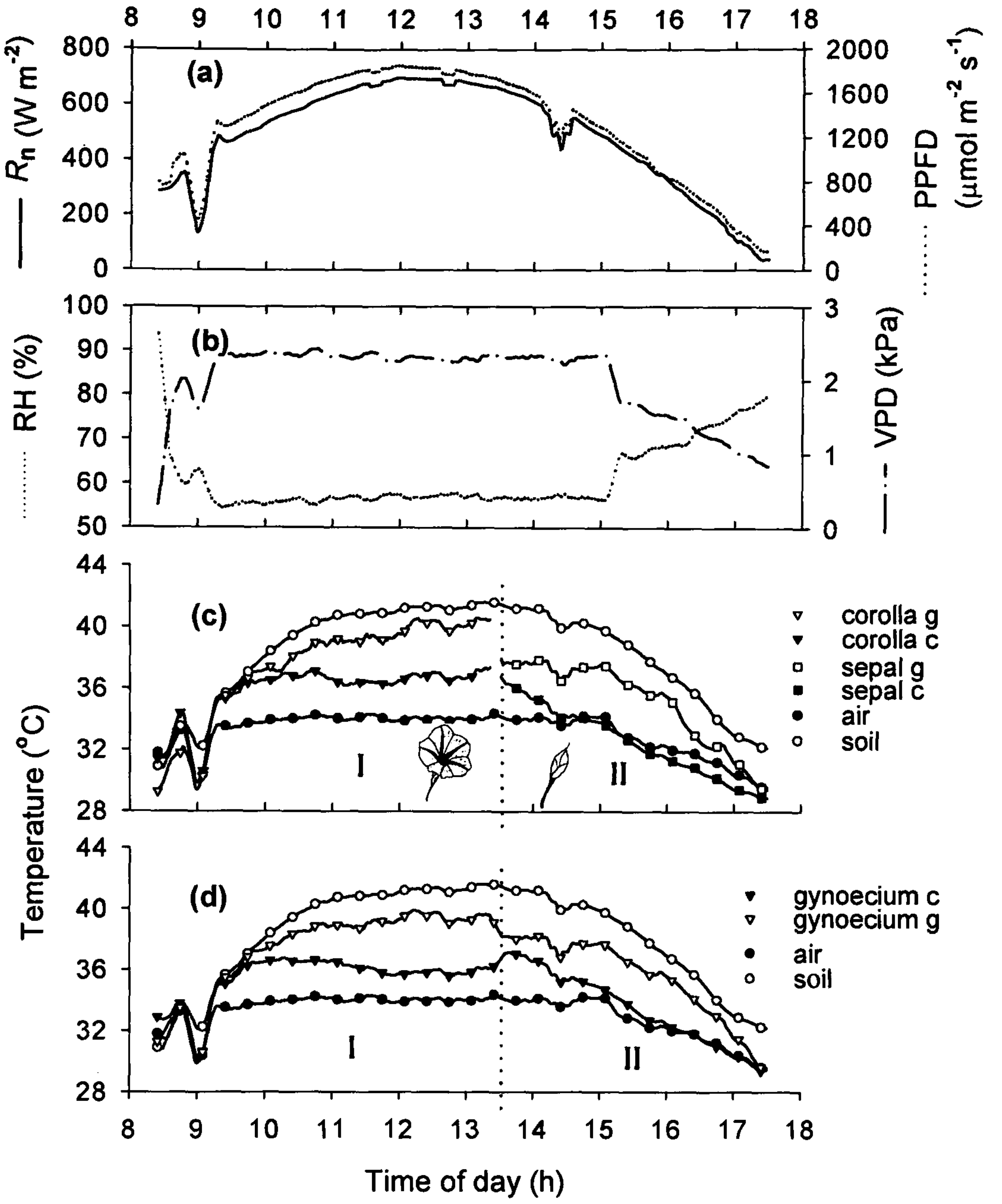


Figure 5.5 Temperature and microclimate of *Ipomoea pes-capre* on the 11th of November 1997. (**a**) Net radiation, R_n (W m^{-2}) and photosynthetic photon flux density, PPFD (µmol m^{-2} s^{-1}); (**b**) Relative humidity, RH (%) and air vapour pressure deficit, VPD (kPa); (**c**) Phase I starts when the flowers open, and ends when the corollas collapsed. The symbols represent temperatures of soil (open circle), greased corollas (open triangle, corolla g), control corollas (filled triangle, corolla c), and air (filled circles). Phase II started after corollas were removed and the temperature of the surface of the sepals was measured instead. The symbols represent temperatures of soil (open circle), greased sepals (open square, sepal g), control sepals (filled square, sepal c), and air (filled circles); (**d**) The plot is divided into two phases as in the previous figure. The symbols represent temperatures of soil (open circle), gynoecia with greased sepals (open triangle, gynoecia g), gynoecia with non-greased sepals (filled triangle, gynoecia c), and air (filled circles).

the sunny period from 9:20 - 15:00 hr and VPD reached a maximum of 2.4 kPa (Fig. 5.5b). The temperature of the control (ungreased) corollas in full sunlight varied from about 35 to 37 °C whilst the air temperature was around 34 °C (Fig. 5.5c). However the temperature of the greased corollas was higher than the temperature of the control corollas, reaching 42 °C, thus demonstrating evaporative cooling by the ungreased corollas, despite their lack of stomata (Phase I, Fig. 5.5c). After the corollas had collapsed the temperature of the greased and control sepals was measured. Greased sepals were warmer than control sepals (from 2.0 to 4.0 K) despite being more or less parallel to the solar beam, suggesting again the cooling effect of evapotranspiration. The temperature of the gynoecia from flowers with greased corollas and sepals varied from 37 - 41 °C and was higher than the temperature of control flowers (Phase I, Fig. 5.5d). When all the corollas naturally collapsed and were removed at around 13:00 h (Phase II, Fig. 5.5d), the temperature of the gynoecia from control flowers rose from 36 to 37 °C and from greased flowers fell from about 39.5 to 38 °C, suggesting 1) that corollas may focus radiation into the gynoecium, 2) that the presence of normally transpiring corollas lower the temperature of the gynoecium, and 3) that sepals play a major role in cooling the gynoecium by transpiration. Data from many flowers with non-greased (controls) and greased corollas and sepals of *M. borneensis*, *I. pes-caprae* and *I. aquatica* were used to determine the extent to which the gynoecia and corollas were warmer or cooler than the air (known as the excess temperature, K) (Fig. 5.6). Differences between species were observed. The larger flowers of *M. borneensis* developed higher excess temperatures than those of the other two species (Fig.5.6). In this species the excess temperatures of the gynoecia exceeded those of the corollas (Fig. 5.6a, d). Excess temperatures in *I. aquatica* were only slight ($\leq$ 3 °C). The extent to which the flowers (gynoecia and corollas) were warmer or cooler than the air was related linearly to the net radiation absorbed by the flowers (Fig. 5.7a-f). The slope of this relation, *m* (°C W^{-1} m^2), estimates the extent to which temperatures of the flower parts increase above air temperature (excess temperature, K) for every unit of radiation absorbed. Table 5.3 contains

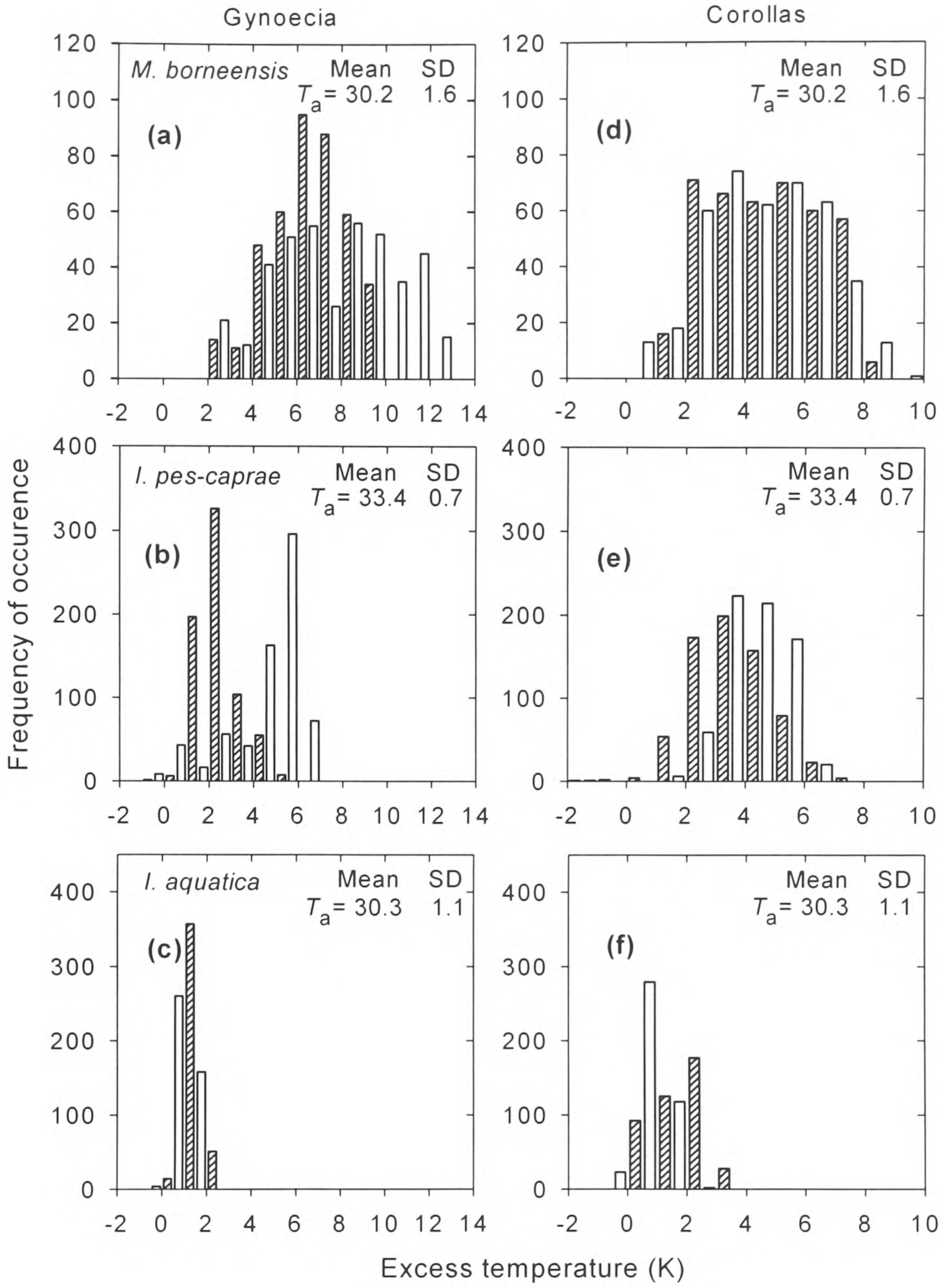


Figure 5.6 Temperatures of gynoecia (a, b, c) and corollas (d, e, f) from field experiments with greased and non-greased (control) corollas and sepals. The term 'Excess temperature' (*x-axis*) refers to the extent to which the tissue is warmer (+) or cooler (-) than the air. The *y-axis* is the number of occurrences based on 1 minute readings from five (*M. borneensis*), six (*I. pes-caprae*) and three (*I. aquatica*) experimental runs. The data shown here are for when PPFD>900 μmol m^{-2} s^{-1}, and from 10 to 13:00 hrs. Open bars represent greased (non-transpiring) corollas and sepals and filled bars represent control (transpiring) sepals and corollas.

the set of values for *m*, and *a* (the intercept, °C) as well as the percent of radiation absorbed with respect to the incoming radiation (measured radiation) and the size of the gynoecia and the diameter of the corolla. There seems to be a tendency for *m* to increase with the size of the flower. Corollas of the three species absorbed little radiation as a consequence of their high shortwave reflectance (Fig. 5.7d – f).

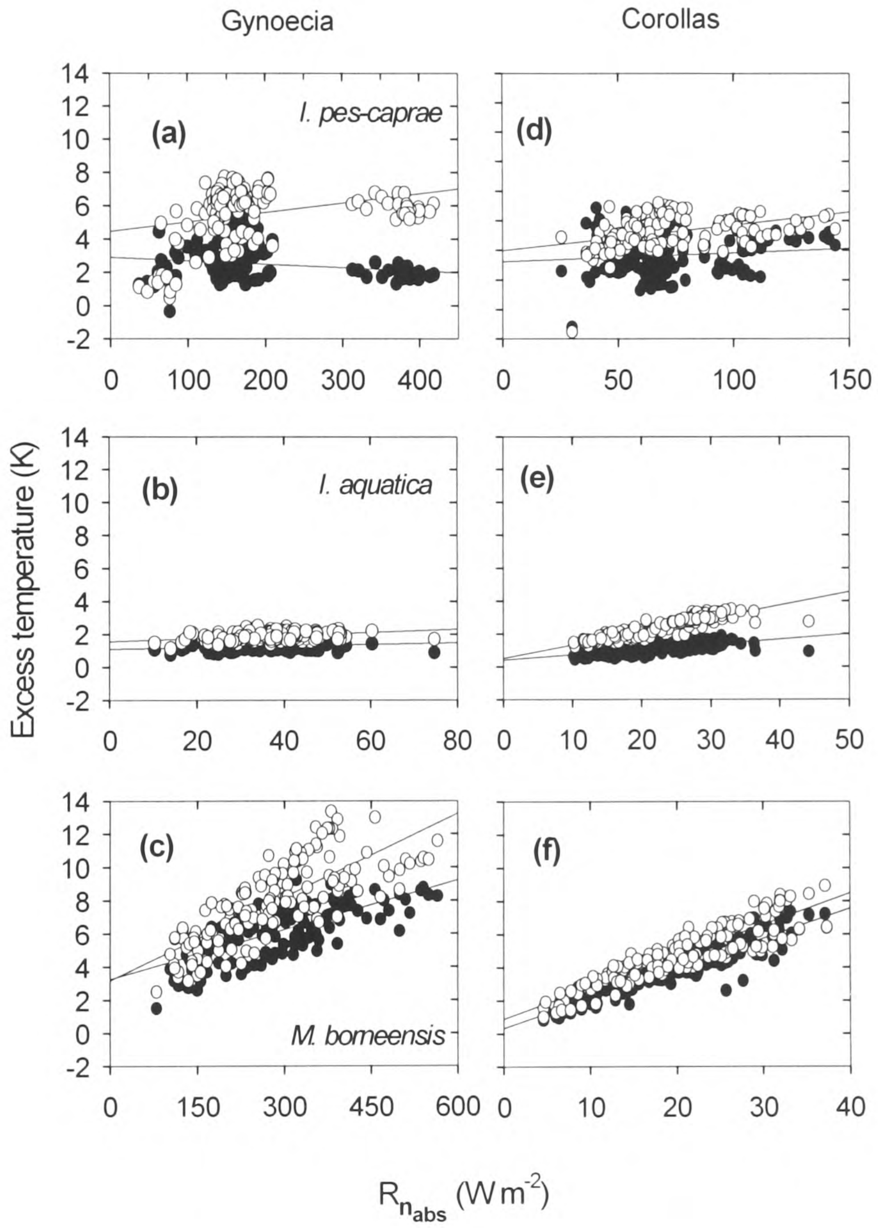


Figure 5.7 Relationship between gynoecium-to-air temperature (**a-c**) and corolla-to-air temperature (**d-f**) and measured net radiation at the site, grouped according to the species: (**a**, **d**) *I. pes-caprae*; (**b**, **e**) *M. borneensis*; and (**c**, **f**) *I. aquatica*. The extent to which the flower part is warmer (+) or cooler (-) than the air here called the 'excess temperature'. Open circles represent greased gynoecia and corollas and closed circle represent control gynoecia and control corollas.

Table 3 Regression analysis of the relationship between excess temperature (K) and net radiation absorbed by corollas and gynoecia (W m^{-2}).

Species		Gynoecia	Corollas
I. pes-caprae	m_c	-0.002	0.006
	a_c	2.9	3.2
	m_g	0.006	0.017
	a_g	4.5	4.0
I. aquatica	m_c	0.005	0.032
	a_c	1.1	0.4
	m_g	0.009	0.081
	a_g	1.6	0.5
M. borneensis	m_c	0.01	0.181
	a_c	3.3	0.3
	m_g	0.016	0.19
	a_g	3.2	0.89

m is the slope (K W^{-1} m^{-2}); a is the intercept (K). The subscripts indicate: c (control) and g (grease).

Energy balance

The estimated energy balance for corollas of *I. pes-caprae* on the 11th of November is shown in Figure 5.8. A similar analysis was made for *I. aquatica* and *M. borneensis*. Differences will be noted below. The calculated net radiation absorbed by corollas and gynoecia closely paralleled the measured net radiation of the site (Fig. 5.8a). The gynoecia absorbed about three times more radiation than the corollas, even when the corollas were in full sunshine and the gynoecia were covered by the corollas. There was also a tendency of E_G to match closely the curve of the calculated E (Fig. 5.8b). In general, wind velocity never exceeded 0.45 m s^{-1} (Fig. 5.8c).

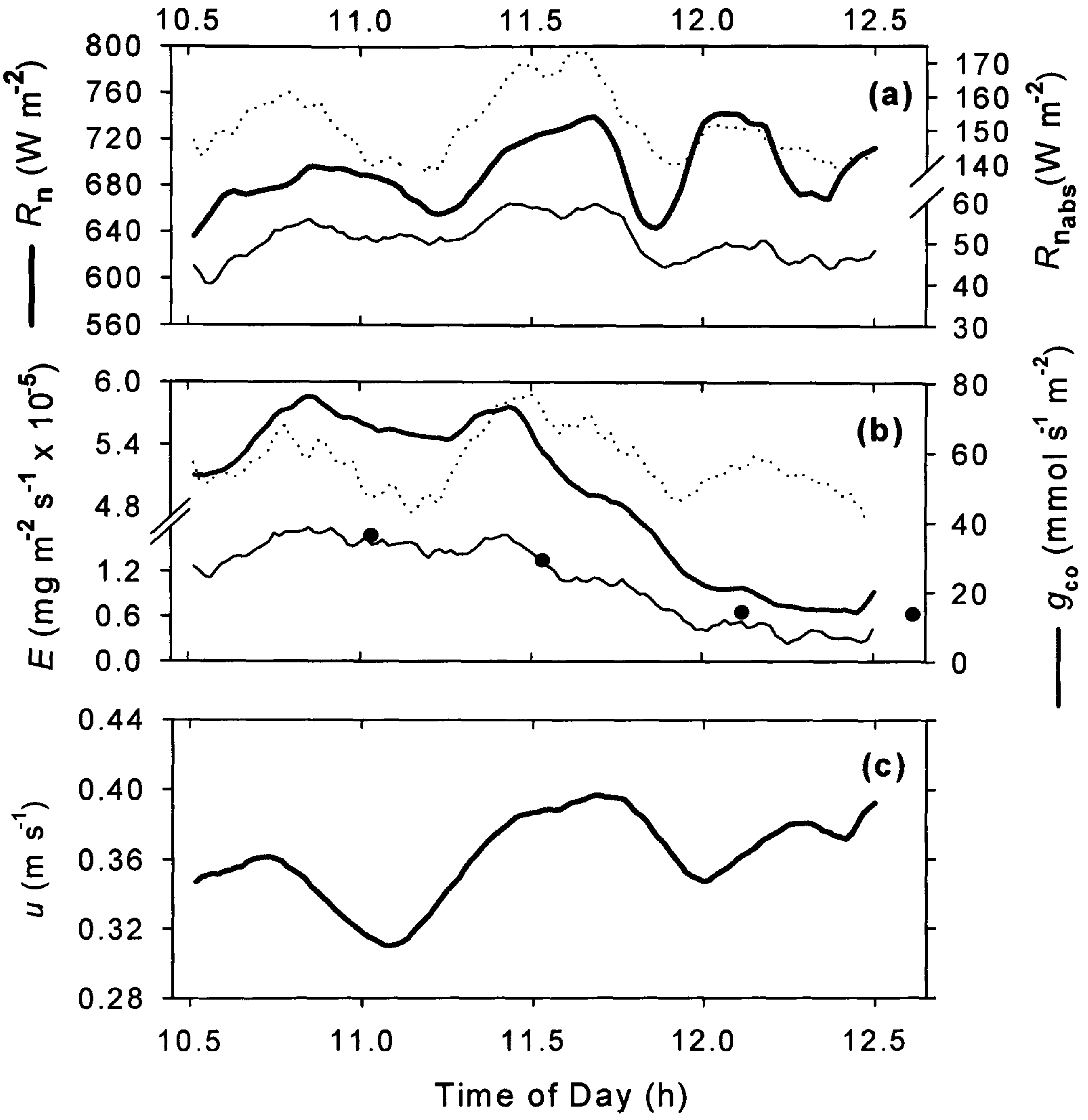


Figure 5.8 Components of the energy balance for *I. pes-caprae* versus time of day (hr). (a) Measured net radiation at the site, R_n (W m^{-2}) (*thick solid line*). Estimated Net radiation absorbed, $R_{n_{abs}}$ (W m^{-2}) by the corollas (*thin solid line*) and gynoecia (*dotted line*) calculated according to {28}. (b) Evaporative flux density, E (mg m^{-2} s^{-1} x 10^{-5}). *Dotted line* represents E of the corollas (*thin solid line*) calculated according to {28}, and filled symbols represent the direct gravimetric measurements of evaporative flux density of corollas, *EG*. The *thick solid line* represents the stomatal conductance of the corollas (g_{co}=mmol s^{-1} m^{-2}). (c) The wind velocity, u (m s^{-1}).

The fraction of net radiation dissipated by evaporation (λE) from the corollas and sepals was very variable (Fig.5.9). For the corollas, it varied from 0.05 to 0.8 for *I. pes-caprae* (Fig. 5.9a), 0.2 to 0.9 for corollas of *I. aquatica* (Fig. 5.9b) and 0.05 to 0.6 for *M. borneensis* (Fig 5.9c). More than half of the net radiation of the gynoecia of *I. pes-caprae* was dissipated by transpiration while less than half was dissipated by the transpiration of sepals of *I. aquatica* (Fig. 5.9e) and *M. borneensis* (Fig. 5.9f). Temperatures of the gynoecium were higher in flowers with greased corollas and sepals than in the control flowers.

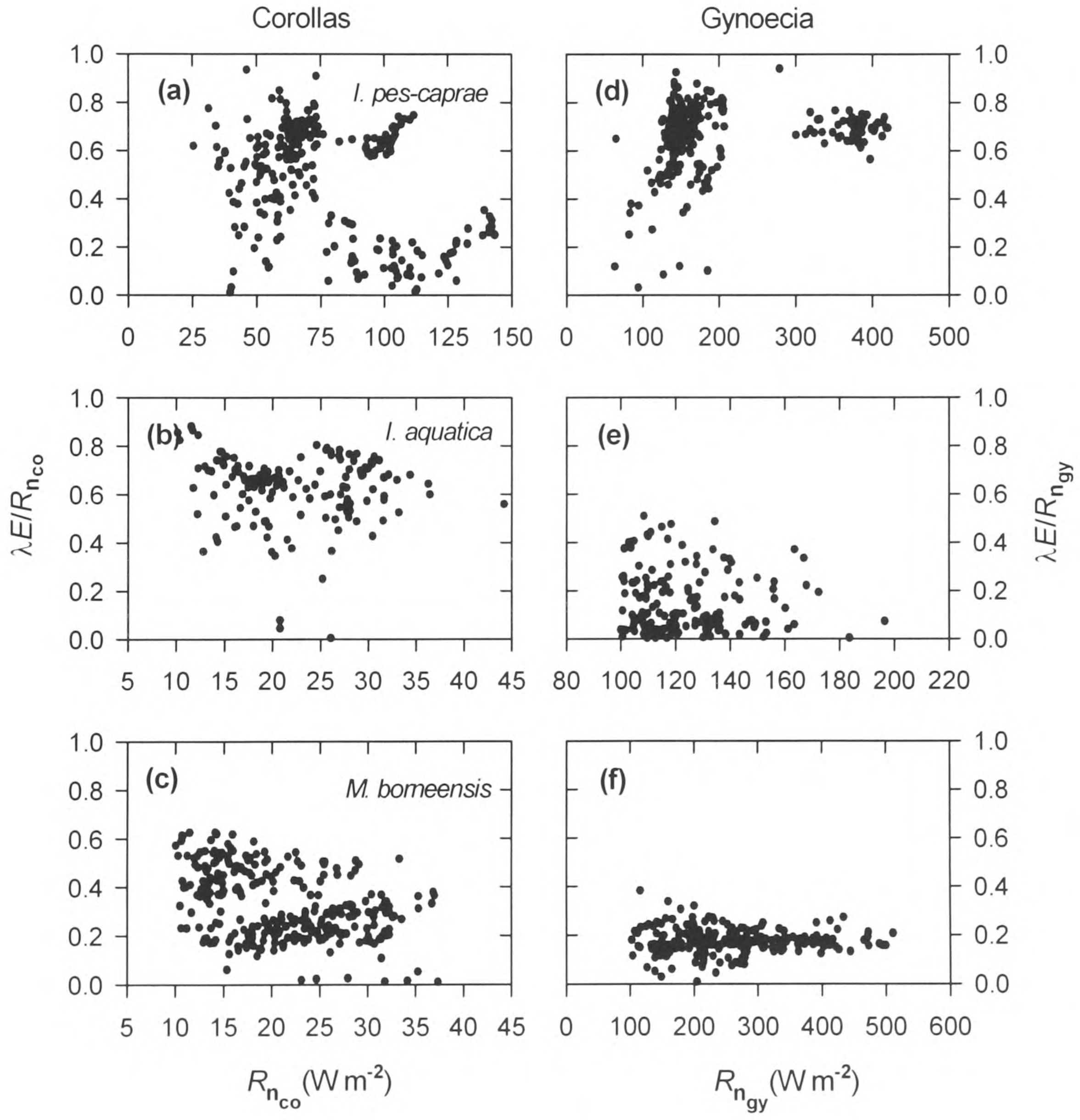


Figure 5.9 The fraction of the absorbed energy of the corolla dissipated as evaporation ($\lambda E/Rn_{co}$) versus Rn_{co} for (a) *I. pes-caprae*, (b) *I. aquatica*, and (c) *M. borneensis*. $\lambda E/Rn_{co}$ is the fraction of net radiation of the corolla that is carried away by latent heat of vaporisation of water. The data includes six experiments of *I. pes-caprae*, five of *I. aquatica* and two for *I. aquatica*.

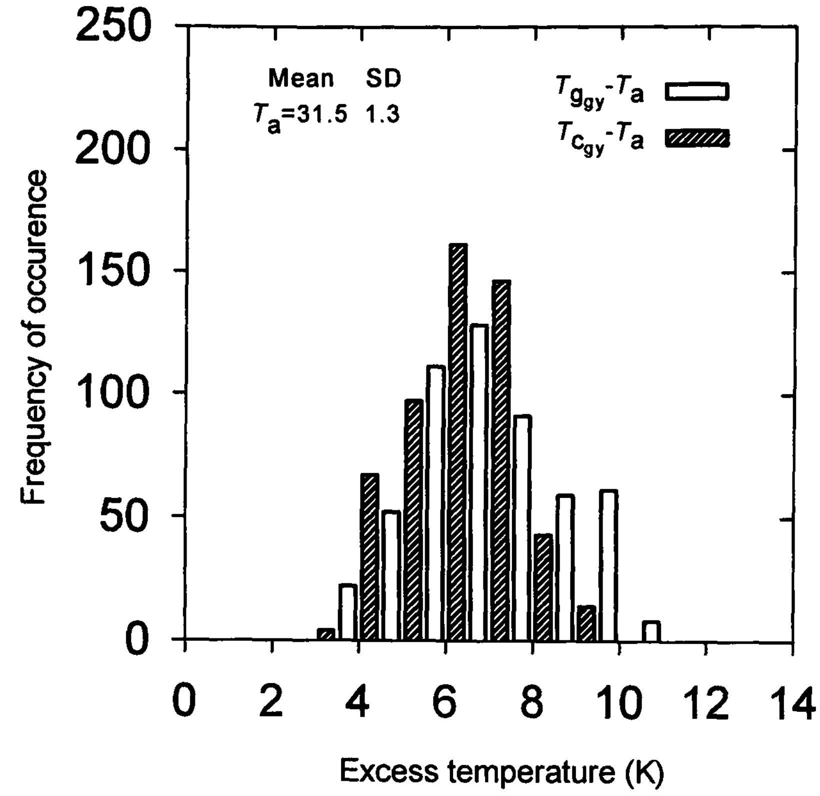


Figure 5.10 Temperatures of the gynoecia of *M. borneensis* from field experiments with greased and non-greased (control) corollas. The term 'Excess temperature' (*x*-axis) refers to the extent to which the tissue is warmer (+) or cooler (-) than the air. The *y*-axis is the number of occurrences based on 1 min readings from three experimental runs (15 greased flowers and 15 control). The data shown here are for when PPFD>900 μmol m^{-2} s^{-1}, and from 10 to 13:00 hrs. Open bars represent greased (non-transpiring) corollas; filled bars represent control (transpiring) corollas.

To asses the real effect of non-transpiring corollas on the gynoecium temperature, a set of new experiments was done. The experiments only included flowers of *M. borneensis* (because of availability of flowers) with greased corollas and normal transpiring sepals. Temperatures and microclimate were measured as described in the Methods section. The results are shown in Figure 5.10. The gynoecium temperature of flowers with greased corollas was higher (1 - 2 K) in the case demonstrating that normal transpiring corollas alone play a role in the cooling of the gynoecia.

Effects of temperature on pollen survival and flower tissue

Sepals and reproductive female parts of *I. pes-caprae* and *M. borneensis* lost vitality at high temperature if exposed for long periods (Fig. 5.11). The tissues of the sepals were affected after 60 min of exposure at temperatures over 37 °C for

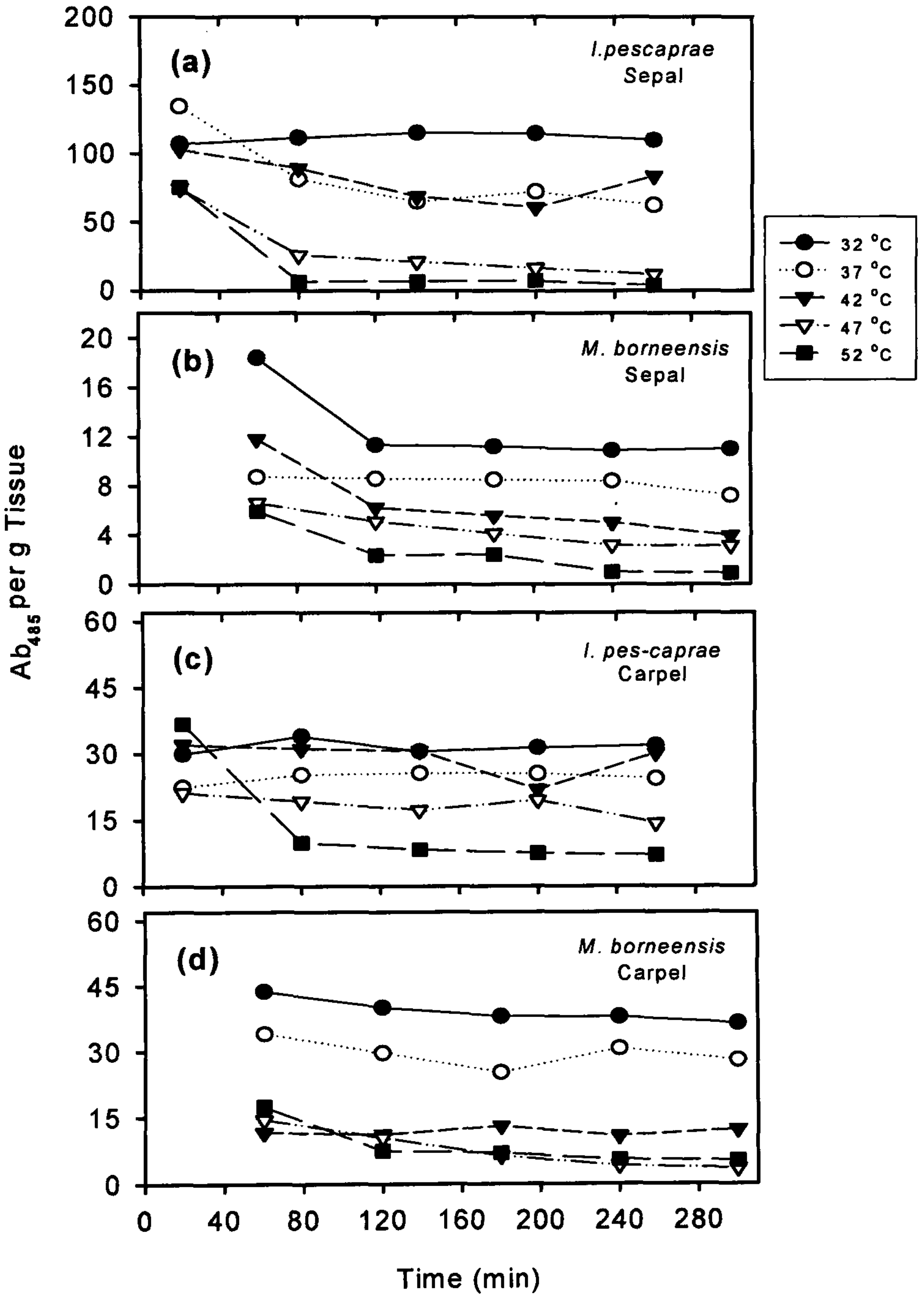


Figure 5.11 Effect of temperature on sepals and female reproductive organs as a function of time for *Ipomoea pes-caprae* and *Merremia borneensis*. The tissue was stained with Tetrazolium (TTC) solution in buffer PH 7.5, after 24 in the dark at 16 °C the TTC was extracted with 95% alcohol. The absorptance at 485 nm of the extracted alcohol (per gram of tissue) was measured with a spectophometer. (**a**) sepal of *I. pes-caprae*; (**b**) sepal of *M. borneensis*; (**c**) carpel of *I. pescaprae* and (**d**) carpel of *M. borneensis*. A high relative absorptance indicates live tissue.

both species (Fig. 5.11a and b) and these temperatures were reached by non-transpiring sepals (Fig 5.5). The reproductive female tissue was also affected by exposure to temperatures over 37 °C (Fig. 5.11c and d). These temperatures where reached by the gynoecia in all the experiments when the sepals and corollas

where prevented from transpirating. These results demonstrate the need for transpiration as a cooling mechanism for the gynoecium. Pollen of *M. borneensis* and *I. pes-caprae* were affected by prolonged exposures to high temperatures (Fig. 5.12). For *I. pes-caprae* (Fig. 5.12a) all pollen died after 20 min at 52 °C while

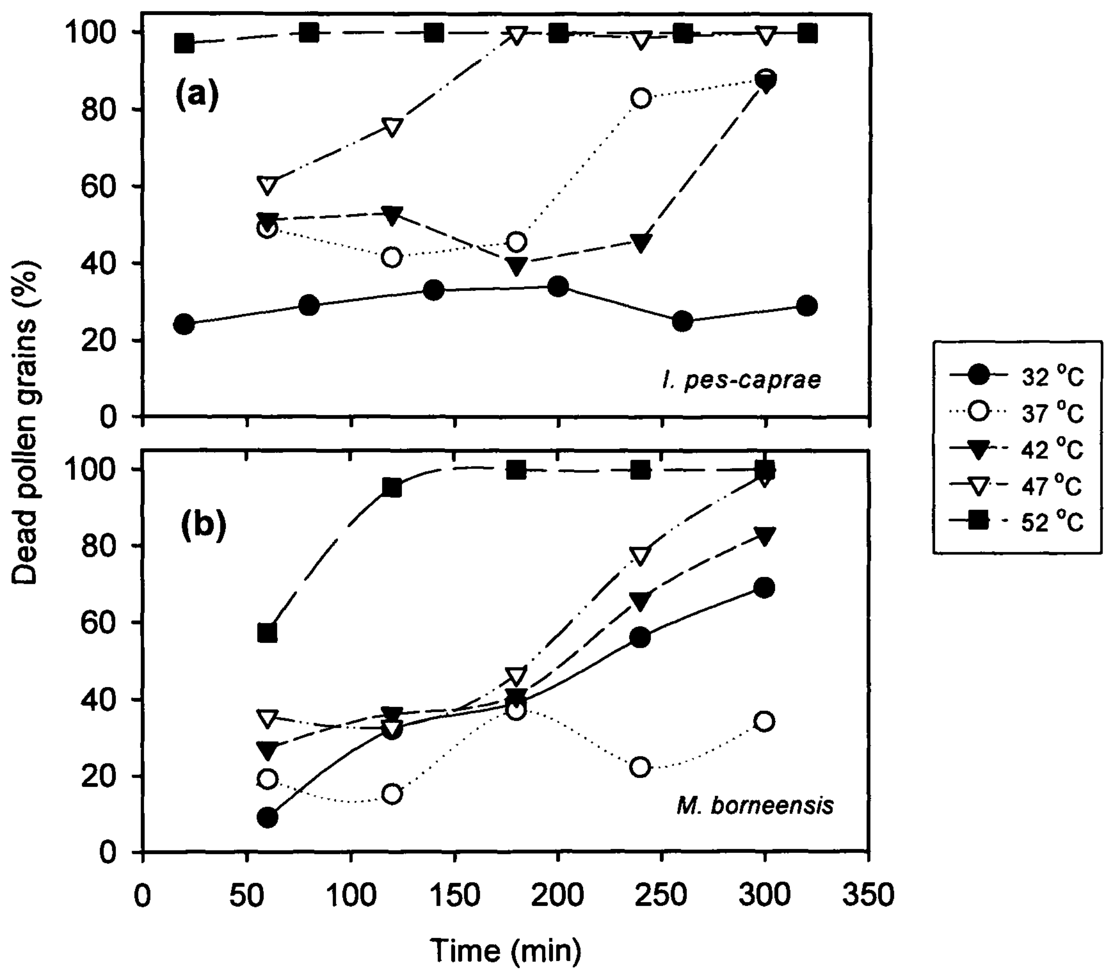


Figure 5.12 Percentage of dead pollen at different temperatures as a function of time. Dead pollen was estimated by counting the number non-stained pollen (white) after being treated with Tetrazolium solution in phosphate buffer Ph 7.5. A grain of pollen was consider viable when it stained pink or red. (a) Pollen from *Ipomoea pes-caprae* and (b) Pollen from *Merremia borneensis*.

pollen of *M. borneensis* died after 2 h of exposure at the same temperature. More than 80 % of the pollen from *I. pes-caprae* died after 3 h at 42 °C and 37 °C. Ambient temperatures of about 32 °C did not have a large effect on pollen viability, although the proportion of dead pollen of *M. borneensis* (Fig. 5.12b) increased at this temperature after 3 h of exposure. This may be the result of pollen germination, that could cause the pollen capsule to be empty by the time of application of TTC. These lethal temperatures (37 to 42°C) were reached and maintained for more than 3 h by non-transpiring flowers (Fig. 5.5).

5.4 Discussion

The evidence from this study is that the characteristic heliotropic trumpet-shape of the convolvulaceous corolla is not merely an advertisement to attract insects but also a parasol or radiation shield to maintain the gynoecium at its functioning temperature. The structure has several useful properties for this role: high reflectance and high conductance to water vapour. At the same time there seems to be a balance between prevention of overheating and reduction of water loss by the corollas. Reduction of water loss may involve decrease of epidermal conductance (i.e. few stomata grouped in the corolla tube), low absorbed radiation (Fig. 5a), reduced evaporative surface (fused petals reduce the exposed surface area) and short-lived flowers (less than one day).

In the present study, stomata were found mainly in the abaxial surface of the corolla tube, and no stomata were found in the outside part of the corolla. However, stomata were numerous on the abaxial surface of sepals, with frequencies that were comparable with those on leaves. For example (Jones 1992) presents a broad range of stomatal frequencies on leaves varying from 0 - 174 on the adaxial surface and from 23 - 600 (stomata mm^{-2}) on the abaxial surface, depending on the phylogenetic scale, environment and photosynthetic pathway of the plants. The frequency of stomata found on the sepals (68 -182 stomata mm^{-2}) is comparable with the frequency on sepals of the strawberry, 100 abaxial stomata mm^{-2} (Blanke 1993), and *Trifolium alexandrium*, 188 stomata mm^{-2} (Shah and Kothari 1975). Several studies show that petals of many flowers are astomatous; strawberry (Blanke 1991), apple (Vemmos and Goldwin 1993), snow-buttercup (Galen, Dawson and Stanton 1993) and 24 flowers from the families *Caryophyllaceae*, *Ranunculaceae*, *Paeoniaceae*, *Brassicaceae*, *Guttiferae*, *Labiatae*, *Asteraceae*, *Dipsacaceae*, *Malvaceae*, *Saxifragaceae*, *Gesneriaceae* and *Onagraceae* (Lipayeva 1989). However stomata do occur on petals of a variety of angiosperm families including *Araceae*, *Hyacinthaceae*, *Convallariaceae*, *Liliaceae*, *Amaryllidaceae*, *Nymphaeaceae*, *Papaveraceae*, *Brassicaceae*, *Oleaceae*, *Menyanthaceae*, *Scrophulariaceae*, *Campanulaceae*, *Asteraceae*, *Orchideaceae* and *Convolvulaceae* (Hew, Lee and Wong 1980; Lipayeva 1989; Shah and Gopal 1969; Watson 1961) but in the majority of cases the suggestion is that the stomata are non-functional. The morphology of stomata in sepals and corollas has been long used as a taxonomic criterion (Watson 1961).

There are few cases in which petals have acquired a role (morphological or physiological) other than the attraction of pollinators. Green cap or corolla of the grape inflorescence, *Vitis vinifera* (Blanke 1989), green fused sepal/petal of the avocado inflorescence, *Persea america* (Blanke and Lovatt 1993) and pink petals (containing chloroplasts) of *Petunia hybrida* (Weiss, Shomer-Ilan, Vainstein et al. 1990) contribute significantly to photosynthesis of the plant. Petals of some alpine flowers are shaped as a paraboloid antenna that focuses radiation into the gynoecium and are though to have positive effect on pollination, fertilisation and fruit development (Corbett, Krannitz and Aarssen 1992; Hocking and Sharplin 1965; Kevan 1975).

The corolla of the species studied here is remarkably short lived, so the radiation shielding does not apply in the afternoon; however the sepals then close around the gynoecium and have the function of cooling the gynoecium by evapotranspiration. It may be expensive in terms of water and carbon components to maintain the corollas for a longer period; it has been suggested that floral longevity is an adaptation that balances the rates of pollen receipt and removal against the cost of floral maintenance (Ashman and Schoen 1994). Studies on the avocado inflorescence *(Persea americana)* (Whiley, Chapman and Saranah 1988) and apple cv Cox's Orange Pippin (Hamer 1986) revealed that there is an increase in transpiration associated with flowering, making flowering particularly expensive in the use of water. Therefore these short lived corollas may be expensive in terms of water loss (E varied from 0.3 - 1.8 mg m^{-2} s^{-1} x 10^{-5}; comparable with rates of water loss by other flowers). For example the fused sepal/petals of the avocado flower transpired 21.6 - 23.4 mg H_2O m^{-2} s^{-1} (Blanke and Lovatt 1993) and strawberry sepal 3.6 - 9.0 mg H_2O m^{-2} s^{-1} (Blanke 1993).

The epidermal conductance to water vapour of the corollas and sepals (20 -80 mmol s^{-1} m^{-2}) were comparable with those of *Ficus* inflorescences (figs) which varied from 20 -380 mmol s^{-1} m^{-2} (Patiño et al. 1994) and leaf conductance of many groups of plants for example succulents, evergreen conifers, deciduous woody plants, herbs from shade habitats, desert and steppe-shrubs and wild graminoids (Jones 1992).

Studies on flowers showed that high temperature and high solar radiation exert detrimental effects on buds, flowers, pollination and fertilisation (Corbet, Willmer, Beament et al. 1979; Cruden, Kinsman, Stockhouse II et al. 1976; Martinez del Rio and Burquez 1986; Rao, Jain and Shivanna 1992; Tanner and Goltz 1972; Totland 1999). However, plants have adaptive mechanisms to overcome high temperatures and high solar radiation on flowers. For example, easterly movements of *Helianthus annuus* reduce heat load of heads, being an advantage for fertilisation (Lang and Begg 1979); figs regulate temperature by transpiration and thus maintain suitable low internal tissue temperatures for the development of the pollinator wasps. Furthermore temperature increases of a few degrees above ambient are enough to induce mass mortality in the wasps (Patiño et al. 1994).

The data from this study on pollen and tissue survival at high temperatures suggest that the reproductive apparatus may have a high vulnerability to thermal damage. There is a considerable body of literature on this subject in relation to crops (Hirokazu, Utsunomiya and Sakuatani 1998); (Kenji and Ikuox 1999). Most authors have demonstrated rather similar responses to temperature to those shown here, and it may be concluded that the reproductive process of plants are often more temperature sensitive than vegetative processes such as photosynthesis and respiration. In the present study it has been demonstrated that the convolvulaceous corollas have adaptive significance in high solar radiation environments, being physiological and structurally equipped to prevent overheating of the gynoecium, as well as fulfilling their more obvious role of attracting pollinators. It may be that many other flowers, in diverse taxonomic groups, also serve to regulate temperature of the reproductive processes, thus ensuring reproductive success in extreme environments.

5.5 Summary

1. The temperatures of flowers of *Ipomoea pes-caprae* ssp *brasilensis*, *Ipomoea aquatica* and *Merremia borneensis* were studied in bright sunshine, to determine the role of corollas and sepals in cooling the gynoecium. Corollas and sepals were prevented from transpiring by greasing to investigate the effect of evaporation on cooling.

2. In the exposed natural habitats of these flowers the maximum temperatures of air and soil were high (32 and 42 °C respectively) and corolla, sepal and gynoecium temperatures were often intermediate.

3. Despite being almost astomatous, significant evaporative cooling was observed in the corolla. Between 20 and 80 % of the energy absorbed by the corollas is dissipated as evaporation.

4. The sepals were stomatous and their evaporative cooling was very important in reducing the temperature of the gynoecium.

5. There is evidence that the heliotropic corolla has a parasol effect which prevent overheating of the gynoecium

Chapter 6

The ecological role of orientation in tropical convolvulaceous flowers

6.1 Introduction

Heliotropism of flowers, the movement of flowers to face the sun, has been observed in alpine, arctic, subarctic, and temperate regions (Hocking and Sharplin 1965; Kevan 1972; Kevan 1975; Kjellberg, Karlsson and Kerstensson 1982; Krannitz 1996; Kudo 1995; Lang and Begg 1979; Smith 1975; Stanton and Galen 1989; Totland 1996). These regions are characterised by low temperatures and a short growing season for plant growth and reproduction.

Figure 6.1 Flowers of *Merremia borneensis* (left) growing at the edge of the forest in Brunei and *ipomoea pes-caprae* (right) growing on the beach at Meragang. This picture suggest that these flowers are oriented in a common direction.

Some explanations have been proposed for the occurrence of heliotropism in flowers. (1) The pollinator attraction hypothesis (Hocking and Sharplin 1965, Hocking 1968, Smith 1975, Kudo 1995) holds that the warmth associated with full insolation of the flower is a direct reward for pollinators. (2) The growth promotion hypothesis (Kevan 1972, 1975, Kjellberg et al. 1982, Young 1984, Kudo 1995, (Kjellberg et al. 1982) assumes that effective absorption of solar energy and the consequent rise in temperature has a favourable effect on pollen germination, growth of the pollen tube and seed production. (3) The reduction of flower temperature (Lang and Begg 1979).

All processes in plants are affected by temperature, but particularly growth, respiration and reproduction (pollination, fertilisation, and seed development (Kudo 1995). Reproduction is mostly regulated by the physiology of the flower consequently floral temperature has a strong influenced in these processes.

In Brunei, a tropical country, flowers of *Ipomoea pes-caprae* the Beach Morning Glory and *Merremia borneensis* (Convolvulaceae), appeared to face the sun (personal observations). *I. pes-caprae* is usually found along the coast on beaches and sandy isolated patches within 500 m from the sea shore. *M. borneensis* in some places is found as a sympatric species with *I. pes-caprae*, as well as along roads, disturbed and grassy areas or in the canopy of secondary vegetation (Fig. 6.1). The blooming period of these flowers is short, lasting less than one day of exposure to the maximum direct solar radiation.

The purposes of the work in this chapter were to investigate whether *I. pes-caprae* and *M. borneensis* are indeed heliotropic flowers, and if so, to explain the ecological relevance of the phenomenon. This involved measurement of the orientation of flowers and also some experimental manipulations to examine the effects of corollas and flower angle (orientation and inclination) on temperatures of the gynoecium.

6.2 Methods

6.2.1 Plant material

This study was carried out on flowers of *Merremia borneensis* Merr. (Fig. 5.2a) and *I. pes-caprae* (L.) Sweet *ssp brasilensis* (L.) Oostr. (Fig. 5.2b), two species belonging to the family Convolvulaceae. The description of the species is presented in detail in the preceding chapter, section 5.2.1.

6.2.2 Study site

This study was conducted in different locations along the East coast of the Island of Borneo, including Brunei Darussalam and Sarawak, Malaysia. The sites were selected because they offered maximum exposure to the sun and availability of the species.

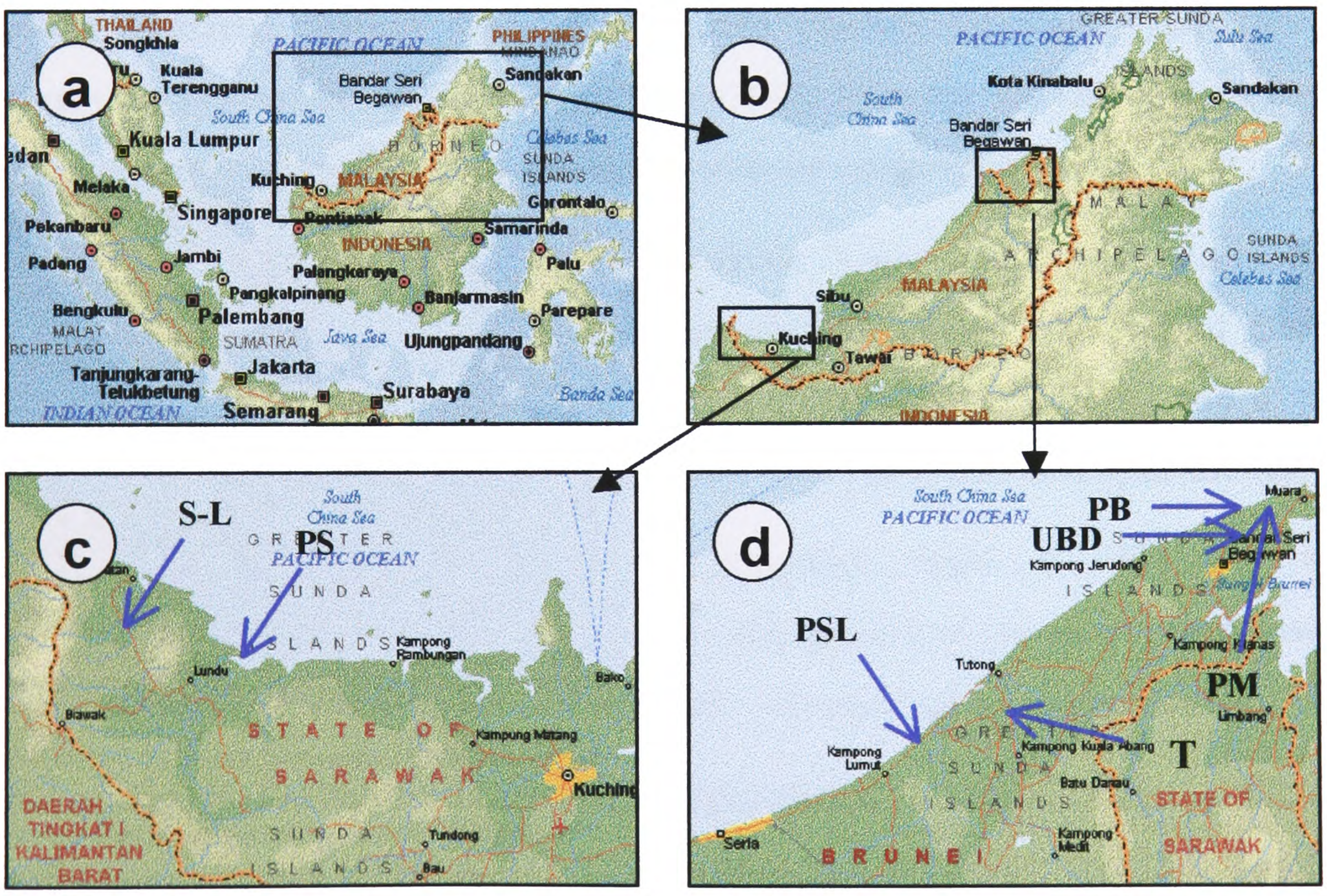


Figure 6.2 The approximate location of the study sites along the coast of Brunei and Sarawak. (**a**) Borneo Island in South East Asia; (**b**) the west coast of Borneo; (**c**) the coast in Sarawak. S-L = the road between Sematan and Lundu, PS = Pantai Siar; and (**d**) the coast of Brunei. PSL = Pantai Sungai Liang, T = Tutong, UBD = University Brunei Darussalam, PB = Pantai Berakas, and PM = Pantai Meragang. (Source of maps: (Expedia.com® 2000)).

Flowers of *M. borneensis* were studied at the main entrance to the campus of the Universiti Brunei Darussalam (4° 58.6' N 114° 53.8' E, elevation 2 m), two sites in the Tutong district, Brunei in January 1999 and one site along the road between Lundu and Sematan, Sarawak, Malaysia in September 1998 (Fig. 6.2c & d).

Flowers of *I. pes-caprae* were studied at a sandy area located on the campus of the Universiti Brunei Darussalam, (114° 53.8' E 4° 58,6' N, elevation 2m) and on different beaches along the west coast of Borneo including Pantai Meragang, Pantai Berakas, Pantai Sungai Liang in Brunei Darussalam (Fig. 6.2d) and Pantai Siar, Lundu, Sarawak, Malaysia (1° 40' N 109° 52' E) (Fig. 6.2c) in November and December 1997, August, September, December 1998 and in January 1999.

6.2.3 Field measurements

Temperature and microclimate

Different experimental treatments of flowers were used: intact flowers, flowers with corollas removed, flowers with the style and stamens removed, shaded flowers, flowers constrained facing the sun and facing away from the sun and greased flowers to restrain evapotranspiration.

Figure 6.3 Experimentally oriented flower of *I. pes-caprae*. To constraint the flower it was attached from the flower stalk to a wire (*W*) stuck in the ground using ordinary cotton sewing thread (*CT*).

Because it was not possible to measure the temperature of all type of flowers in one experimental run, there were two different types of experiments:

Experiment type I: Flowers with corolla removed versus intact corolla (only flowers of *M. borneensis*).

Experiment type II: Flowers maintained in their natural position, facing the sun versus flowers constrained 180° to the normal orientation, facing away from the sun (flowers of *I. pes-caprae* and *M. borneensis*). 10-19 flowers were used per experiment. Half of them were mechanically constrained by attaching the peduncle to an arch made of wire and inserted in the ground at about 10 cm from the flower (Fig. 6.3). Half of the constrained and half of the natural oriented flowers were greased with silicone gel to prevent transpiration.

Temperatures were measured using copper-constantan thermocouples (0.5 mm diameter), which were calibrated against an Hg thermometer. The thermocouples were inserted in the centre of the gynoecium (T_{gy}), in the corolla (T_{co}), or in the interior (T_{in}) (according with the type of experiment) of each flower (Fig. 6.4). Measurements of microclimatic variables and flower temperature followed the procedure described in chapter 5 otherwise it is specified in the text.

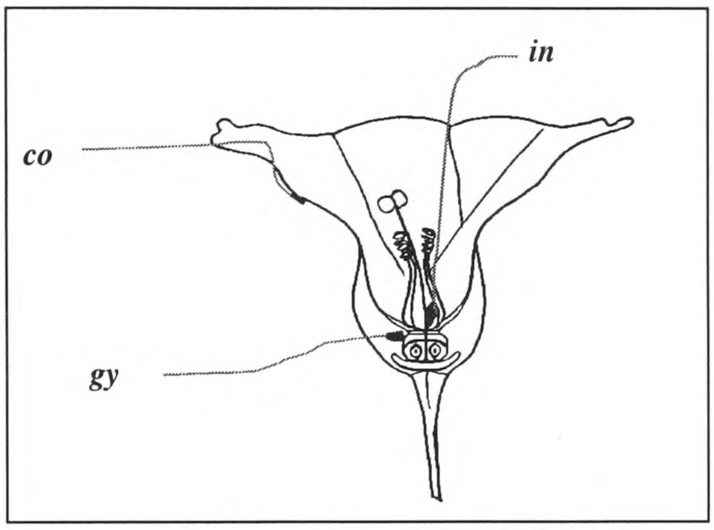


Figure 6.4 Schematic representation of a flower with the location of the thermocouples in the corolla (*co*), gynoecium (*gy*) and interior (*in*).

Inclination of the flowers i.e. the angle from the horizontal, was measured with a clinometer (SF-02920, Suunto, Espoo, Finland). Orientation was measured with a compass (MC-1, Suunto, Finland). To assess the precision of the inclination and orientation measurements a test was performed. 100 readings were taken from the same flower. The variance was calculated and the standard error was estimated as ± 0.3 degrees for the orientation and ± 0.14 for the inclination.

Flower azimuth and inclination angle were tested using Rayleigh's test (Forseth and Ehleringer 1982). The null hypothesis for azimuth was that the flowers came from a population uniformly distributed around a circle i.e. no direction was preferred. If the null hypothesis was rejected, the conclusion was that there was a mean population direction i.e. a particular direction was preferred. The null hypothesis for the inclination angle (the angle from the horizontal) was that the population had a mean angle of 0° (i.e. the flowers were vertical, corollas facing the zenith).

To establish if the flowers track the sun during the day, differences in orientation and inclination between hours were tested with Watson's U^2 test (Forseth and Ehleringer 1982):629 a two-sample nonparametric test. The null hypothesis for flower azimuth was that the two samples came from two populations that had the same direction. If the null hypothesis was rejected, the conclusion was that the flowers had different orientation at different times of the day. The null hypothesis for the flower inclination angle was that the two samples had the same angle from the horizontal.

Insect visitation

Insects (Hymenoptera) visiting the different treated flowers were counted for 10-60 min periods over several days. Only when the insect remained in the flower for more than 3 s was it counted.

For *M. borneensis*, all the observations were done at the University of Brunei campus from plants climbing around some bushes. For each day of observations two groups of flowers were selected. One group was from plants growing behind the bushes (not facing the sun) and the second group was from plants growing in the front part of the bush (facing the sun). The plants behind the bushes had no flowers, so excised flowers were placed where they might have occurred (over the course of measurements they did not wilt). Within these two groups there were different treated flowers: intact flowers, flowers with corollas removed, flowers with the style and stamens removed. To determine if the number of visiting insects per flower was the same for both conditions, the differences in mean number of insects per flower were examined with a One-way Analysis of Variance (Minitab 12.3).

For flowers of *I. pes-caprae*, observations of insect visitation were done in Meragang beach. In this case several flowers were tethered east and the same number were tethered west.

6.3 Results

Determination of flower heliotropism

Figures 6.5, 6.6 and show polar plots with the position (azimuth and inclination) of flowers for different dates and locations. In all of the sampled populations it was possible to calculate a mean direction suggesting that these convolvulaceous flowers were not oriented uniformly in all directions. Table 6.1 presents a summary of the mean azimuth and inclination angles of the flowers for the two species, dates and locations. In the majority of cases the flowers were pointing in a 'preferred' direction. However, in two of the samples (Highway on the 23rd of August and Sungai Liang on the 18th of December) for *I. pes-caprae* the null hypotheses was accepted suggesting that in some locations or times of the year the flowers were not oriented in a mean direction or they were oriented uniformly in all directions.

The 95% confidence interval for *a'* (the calculated mean inclination angle) in all the cases did not contain the hypothesized mean of 0°, and so the null hypothesis was rejected, meaning that the flowers were not facing the zenith. The mean inclination angle for *I. pes-caprae* varied between 21 and 45°, and for *M. borneensis* between 20° and 46°.

Table 6.1 Mean of the azimuth and inclination angles on different dates and locations for flowers of *Ipomoea pes-caprae* and *Merremia borneensis*. The null hypothesis for azimuth was that the flowers came from a population uniformly distributed around a circle. The null hypothesis for the inclination angle was that the populations had a mean inclination of 0°.

Species	**Dates**	**Location**	***n***	***Az***	***P***	***Ho***	***a'***
Ipomoea pes-caprae	*20 Aug-98	UBD	60	9±15	<0.001	r	29±4.0
	23-Aug-98	Highway	66	356±71	<0.117	a	28±1.4
	23-Aug-98	Meragang	108	36±12	<0.001	r	39±2.7
	*11-Nov-97	UBD	16	190±39	<0.001	r	42±2.6
	*3-Dec-97	UBD	16	161±33	<0.001	r	34±1.0
	*6-Sep-98	Meragang	95	348±22	<0.009	r	26±5.1
	26-Sep-98	Lundu	99	4±10	<0.002	r	36±10.2
	12-Dec-98	Berakas	23	89±36	<0.005	r	37±4.1
	17-Dec-98	Meragang	38	223±39	<0.008	r	40±4.8
	18-Dec-98	Sungai Liang	33	255±	<0.154	a	40±4.2
	19-Dec-98	Berakas 1	37	188±47	<0.029	r	34±2.8
	19-Dec-98	Berakas 2	46	232±34	<0.002	r	39±4.5
	25-Jan-99	Berakas 2	61	239±39	<0.001	r	24±3.4
	25-Jan-99	Berakas 3	32	213±23	<0.001	r	28±3.5
Merremia borneensis	26-Sep-98	Lundu 1	83	84±22	<0.001	r	46±3.3
	26-Sep-98	Lundu 2	51	177±49	<0.0352	a	41±3.6
	7-Jan-99	UBD	278	141±5	<0.001	r	42±1.7
	20-Jan-99	UBD	167	116±9	<0.001	r	35±1.5
	20-Jan-99	Tutong 1	23	164±17	<0.001	r	41±5.5
	20-Jan-99	Tutong 2	25	199±28	<0.001	r	20±--

* An asterisk indicates that the date on the table correspond to the grouping of several days: 18, 21, 22 August = 20 Aug, 5, 7 September = 6 Sep, 9, 10, 11, 12 November = 11 Nov, 4,5,6,7,8,9,10 January = 7 Jan, and 19, 20, 22, 23, 24 Jan = 20 Jan. The symbols: ***n*** is the sample size, ***Az*** is calculated mean azimuth ± the 95% confidence interval for the population mean direction, ***P*** the probability, ***Ho*** null hypothesis (r = rejected and a = accepted), ***a'*** is the calculated mean inclination angle ± the 95% confidence interval for the population mean inclination angle. The azimuth was measured from 0-360°, with 0° = north, 90° = east, 180° = south, and 270° = west. The inclination was measured from the horizontal (0-90°), with 0° = a vertical flower with the corolla facing the zenith and 90° an horizontal flower with the corolla facing north, east, south or west.

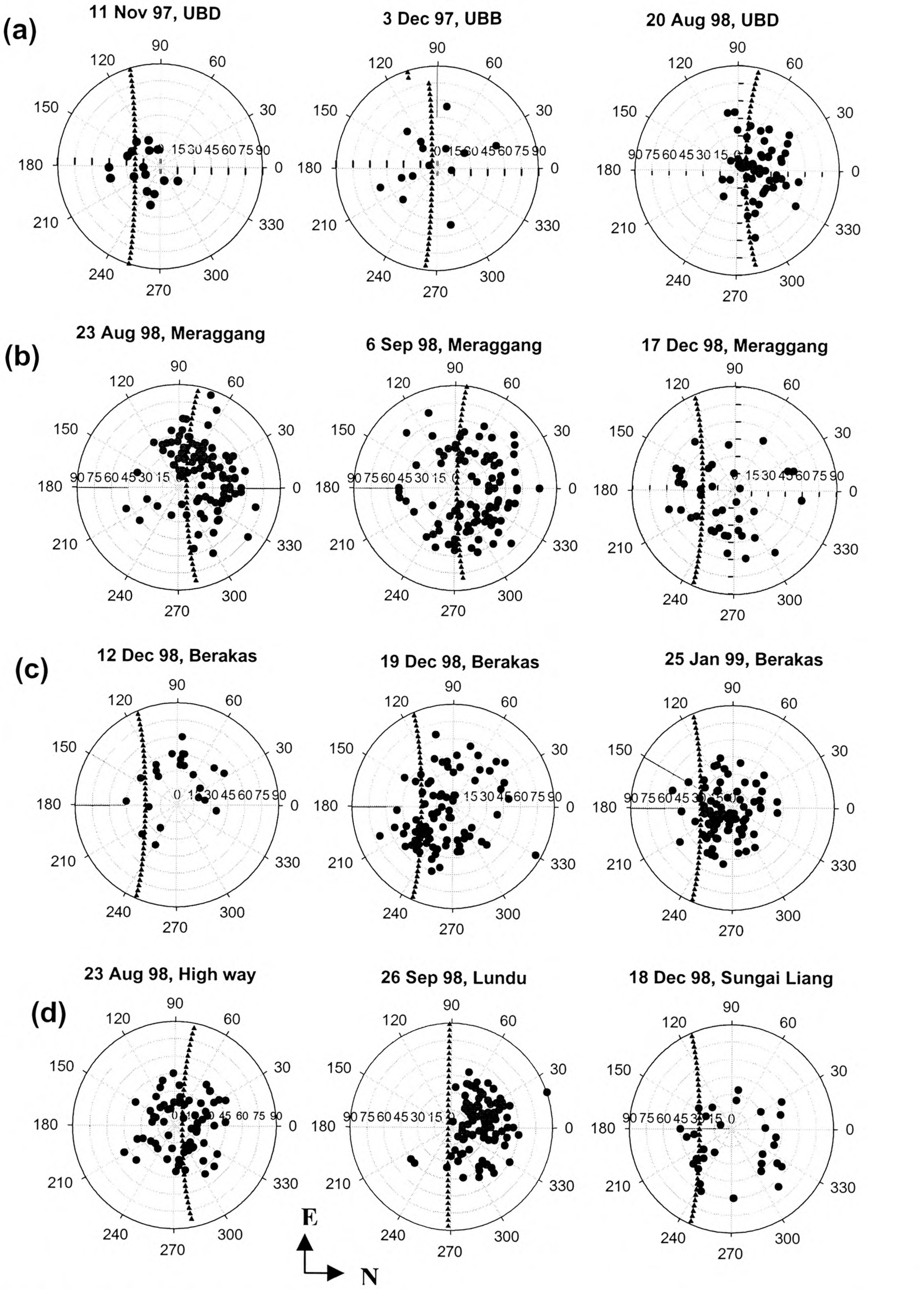


Figure 6.5 Distribution of the orientation of *Ipomoea pes-caprae* flowers for different locations and time of the year. Each filled circle represents the orientation of a single flower described by its inclination angle and azimuth angle. All measurememnts were done between 9:00 and 11:00h. The corollas were shed in the afternoon. The triangles represent the sun-path in that particular day and location (**a**) University Brunei Darussalam campus (UBD); (**b**) Pantai Meragang (**d**) Pantai Berakas; and (**d**) Coastal High way (Brunei), Lundu, Sarawak, Malaysia and Pantai Sungai Liang (Brunei).

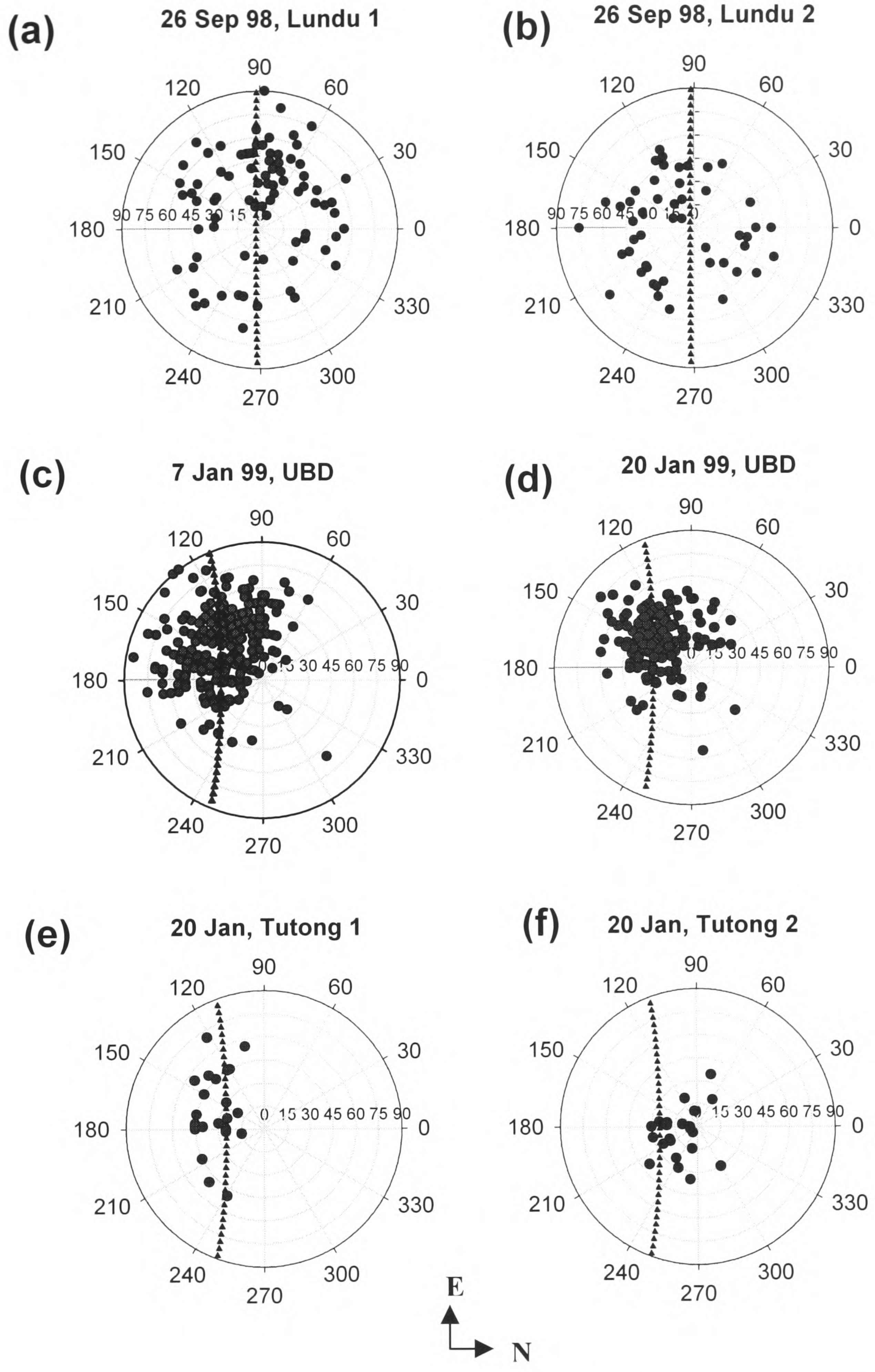


Figure 6.6 Distribution of the orientation of *Merremia borneensis* flowers for different locations and time of the year. Each filled circle represents the orientation of a single flower described by its inclination angle and azimuth angle. All measurements were done between 9:00 and 11:00h. The triangles represent the sun-path in that particular day and location (**a, b**) Lundu, Sarawak, Malaysia; (**c, d**) University Brunei Darussalam campus (UBD) Pantai Meraagan (**e, f**) Tutong District, Brunei.

Solar tracking

To explore the possibility that the flowers were solar trackers, the position (azimuth and inclination angle) of *I. pes-caprae* flowers was measured three times per day for four days. The flowers were oriented to a preferred angle i.e. the null hypothesis for flowers being oriented uniformly in all directions was rejected in all the sampling times and days (Table 6.2). Flowers of *I. pes-caprae* did not track the sun during the day. Table 6.3 presents a summary of the results for the Watson's test for the azimuth and Table 6.4 presents the summary of the results of the Watson's test for the inclination angle for *I. pes-caprae*. The orientation of these flowers appears to be determined by the position of the sun during the year. In the period before the equinox, when the sun was in the northern part of the sky, the flowers tended to point north; but after the equinox, when the sun was in the southern part of the sky, the flower tended to point south.

Table 6.2 Mean flower azimuth and inclination for *I. pes-caprae* on four different dates and at three different hours of the day (except for the 20th of August that only had two sampled hours) on Meragang beach, Brueni. The null hypothesis for azimuth was that the flowers came from a population uniformly distributed around a circle. The null hypothesis for the inclination angle was that the populations had a mean inclination of 0°.

Dates	**Time of day (h)**	***n***	***Az***	***P***	***Ho***	***a'***
18-Aug	7	19	3±36	<0.004	r	20±6.0
	10	22	348±29	<0.001	r	25±5.8
	13	21	8±27	<0.001	r	22±5.9
20-Aug	7	13	348±31	<0.001	r	25±6.1
	12	15	3±27	<0.001	r	27±8.7
21-Aug	7	20	21±33	<0.002	r	29±4.7
	10	16	35±22	<0.001	r	28±7.8
	13	21	32±49	<0.001	r	36±8.9
22 Aug	7	22	3±20	<0.001	r	31±6.2
	10	22	6±23	<0.001	r	34±7.0
	13	22	359±26	<0.001	r	37±7.8

n is the sample size, ***Az*** is the calculated mean azimuth ± the 95% confidence interval for the population mean direction, ***Ho***, null hypothesis whether rejected (r) or accepted (a), ***a'***, calculated mean inclination angle ± the 95% confidence interval for the mean angle. The azimuth was measured from 0-360°, with 0° = north, 90° = east, 180° = south, and 270° = west. The inclination was measured from the horizontal (0-90°), with 0° = a vertical flower with the corolla facing the zenith and 90° an horizontal flower with the corolla facing north, east, south or west.

Table 6.3 Summary of the Watson's U^2 test for azimuth at different sampling dates at different hours of the day for *Ipomoea pes-caprae*. The null hypothesis for flower azimuth was that the two samples came from two populations that had the same direction (or two hours of the day that had the same direction).

Date	*T1-T2*	*N1*	*N2*	*N*	U^2	*P*	*Ho*
18 Aug 99	7 – 10	17	22	39	0.0850	$0.2<P<0.5$	a
	7 –13	17	20	37	0.0492	$0.5<P$	a
	10 – 13	22	20	42	0.0737	$0.2<P<0.5$	a
20 Aug 99	10 –12	13	15	28	0.0573	$0.5<P$	a
21 Aug 99	7 – 10	20	16	36	0.1025	$0.2<P<0.5$	a
	7 –13	20	20	40	0.0462	$0.5<P$	a
	10 – 13	16	20	36	0.1676	$0.1<P<0.05$	a
22 Aug 99	7 – 10	22	22	44	0.1467	$0.2<P<0.1$	a
	7 –12	22	22	44	0.1025	$0.2<P<0.5$	a
	10 – 12	22	22	44	0.1333	$0.2<P<0.1$	a
7 Sep 99	8 – 11	10	10	20	0.0522	$0.5<P$	a

T1** and **T2 are the two populations/hours of the day being tested, ***N1*** and ***N2*** are the sample size of the two populations/hours being tested, ***N*** = *N1+N2*, U^2 is the test statistic, called the Watson U^2, ***P*** is the probability and ***Ho*** is the null hypothesis whether accepted (a) or rejected (r).

Table 6.4 Summary of the Watson's U^2 test for inclination angle at different times of day. The null hypothesis for the flower inclination angle was that the two samples had the same angle from the horizontal.

Date	*T1-T2*	*N1*	*N2*	*N*	U^2	*P*	*Ho*
18 Aug 99	7 – 10	19	22	41	0.1142	$0.1<P<0.05$	a
	7 –13	19	21	40	0.0607	$0.5<P$	a
	10 – 13	22	21	43	0.0308	$0.5<P$	a
20 Aug 99	10 –12	13	15	28	0.0773	$0.2<P<0.5$	a
21 Aug 99	7 – 10	20	16	36	0.1686	$0.1<P<0.05$	a
	7 –13	20	21	41	0.3216	$0.005<P<0.002$	r
	10 – 13	16	21	37	0.1537	$0.1<P<0.05$	a
22 Aug 99	7 – 10	22	22	44	0.0936	$0.2<P<0.5$	a
	7 –12	22	22	44	0.1453	$0.1<P<0.2$	a
	10 – 12	22	22	44	0.0471	$0.5<P$	a
7 Sep 99	8 – 11	10	10	20	0.0267	$0.5<P$	a

T1** and **T2 are the two populations/hours of the day being tested, ***N1*** and ***N2*** are the sample size of the two populations/hours being tested, ***N*** = *N1+N2*, U^2 is the test statistic, called the Watson U^2, ***P*** is the probability and ***Ho*** is the null hypothesis whether accepted (a) or rejected (r).

A useful test for solar tracking is to examine the behaviour of the cosine of the angle of incidence of solar radiation impinging on the flower (cosine of the angle between the sun's direct rays and a normal to the flower corolla, taking into account both azimuth and inclination, equation 2.13). If the flowers were tracking the sun, the cosine of incidence would be close to 1.0 during the whole day. For flowers of *I. pes-caprae*, the value fluctuated from 0.67 in the morning (at low solar altitudes) to almost 0.92 at solar noon, implying a deviation of 48° to 22°.

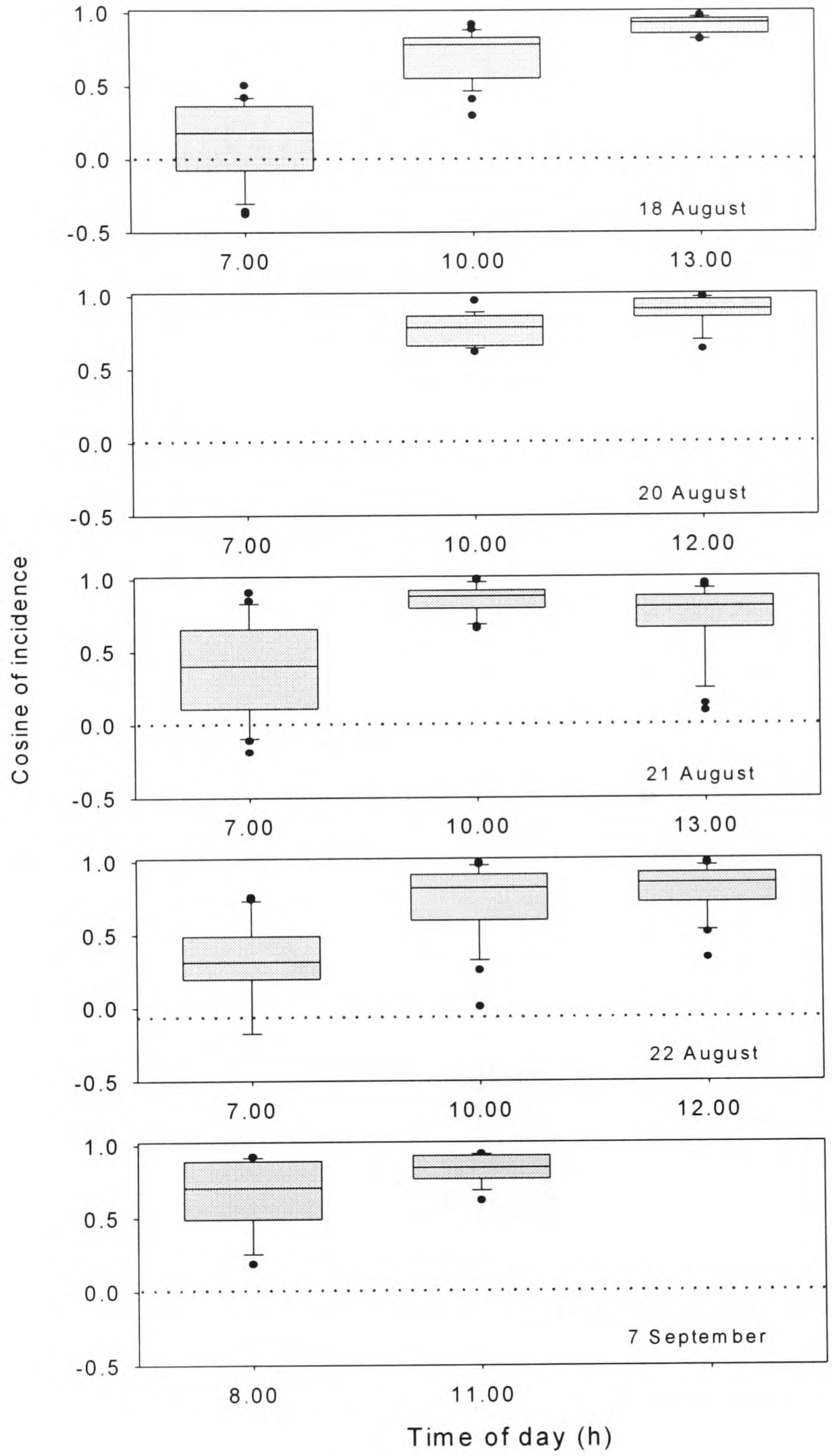


Figure 6. 7 Calculated cosine of incidence of sunlight for flowers of *I. pes-caprae* at different hours of day on five days in 1998. Each bar includes 10-22 flowers. Note that the X-axis is not the same for each case.

(Fig. 6.7). The cosine of incidence for the flowers of *M. borneensis* was close to 1.0 with almost no difference between sampling times (Fig. 6.8). For example the

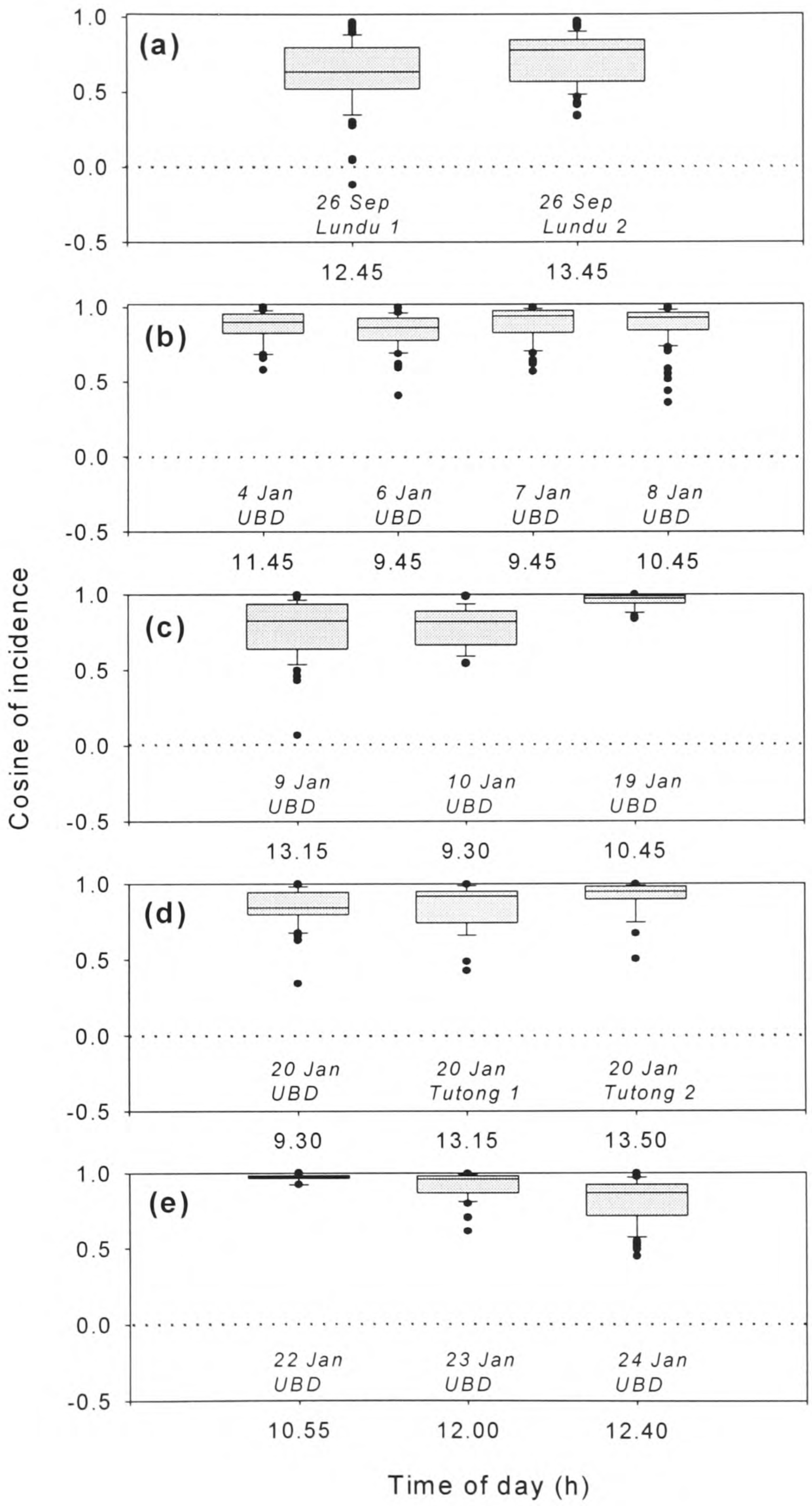


Figure 6.8 Calculated cosine of incidence of sunlight for flowers of *M. borneensis* on different locations and dates. The X-axis indicates the time of the day at which the measurements were done. The number of flowers per location and date varies from 25 to 278. (**a**) Lundu, Sarawak, two locations on the26th of September 1998; (**b** and **c**) At the Universiti Brunei Darussalam (UBD) campus several days in early January 1999; (**d**) UBD, Tutong 1 and Tutong 2 on the 20th of January 1999; and (**e**) UBD three days in late January 1999.

sampling during January at UBD, with sampling between 9:30 and 13:15, the cosine of incidence varied between 0.77 on the 9th (Fig. 6.8c) to 0.97 on the 22nd (Fig. 6.8e). The range was from 0.77 to 0.97, implying a deviation of 40° to 14° from the solar target.

The temperature of the gynoecium was related to the position (determined by the inclination angle and the azimuth) of the flower. Five experimental runs of the experiment type I were performed for *M. borneensis* and all showed the same trend. Only one day (23rd of January 1999) is shown for *M. borneensis* in Figure 6.9. Naturally oriented flowers were cooler (41.0 °C) than constrained flowers (44.2 °C) during the sunniest period (Fig. 6.9a) this suggest that the corolla is like a parasol when the flower faces the sun and without it the solar beam hits the underside of the flower, increasing the temperature of the gynoecium. When the incident radiation was diffused (cloudy periods) the effect of temperature diminished (Figs. 6.9a and c). When flowers were prevented from transpiration (evaporative cooling) but kept in their natural position, there was an increase of the temperature of the gynoecium from 41.0 °C to 47.1 °C relative to the naturally oriented flower in the sunniest period, yet the effect of preventing transpiration alone was less than the effect of changing the orientation (180° from the natural position). The calculated cosine of incidence for the flowers in this experiment indicates that the naturally oriented flowers are almost facing the sun i.e. cosine of incidence close to 1.0 (Fig. 6.9b). The trend of calculated cosine of incidence for the constrained flowers was opposite to the one for naturally oriented flowers and this seems to have an effect on the thermal balance of the flower (Figs. 6.9a and b). Still the gynoecium temperature follows the pattern of PPFD and net radiation during the day (Fig. 6.9c). The minimum humidity was reached at around 13:00 hr and the wind velocity changed according to the PPFD, and the maximum was reached at midday (Fig. 6.9d). In conclusion, the lowest temperatures were recorded in gynoecia of flowers that were not manipulated, the results suggest that both transpiration and orientation play a role in cooling the flower. The patterns of

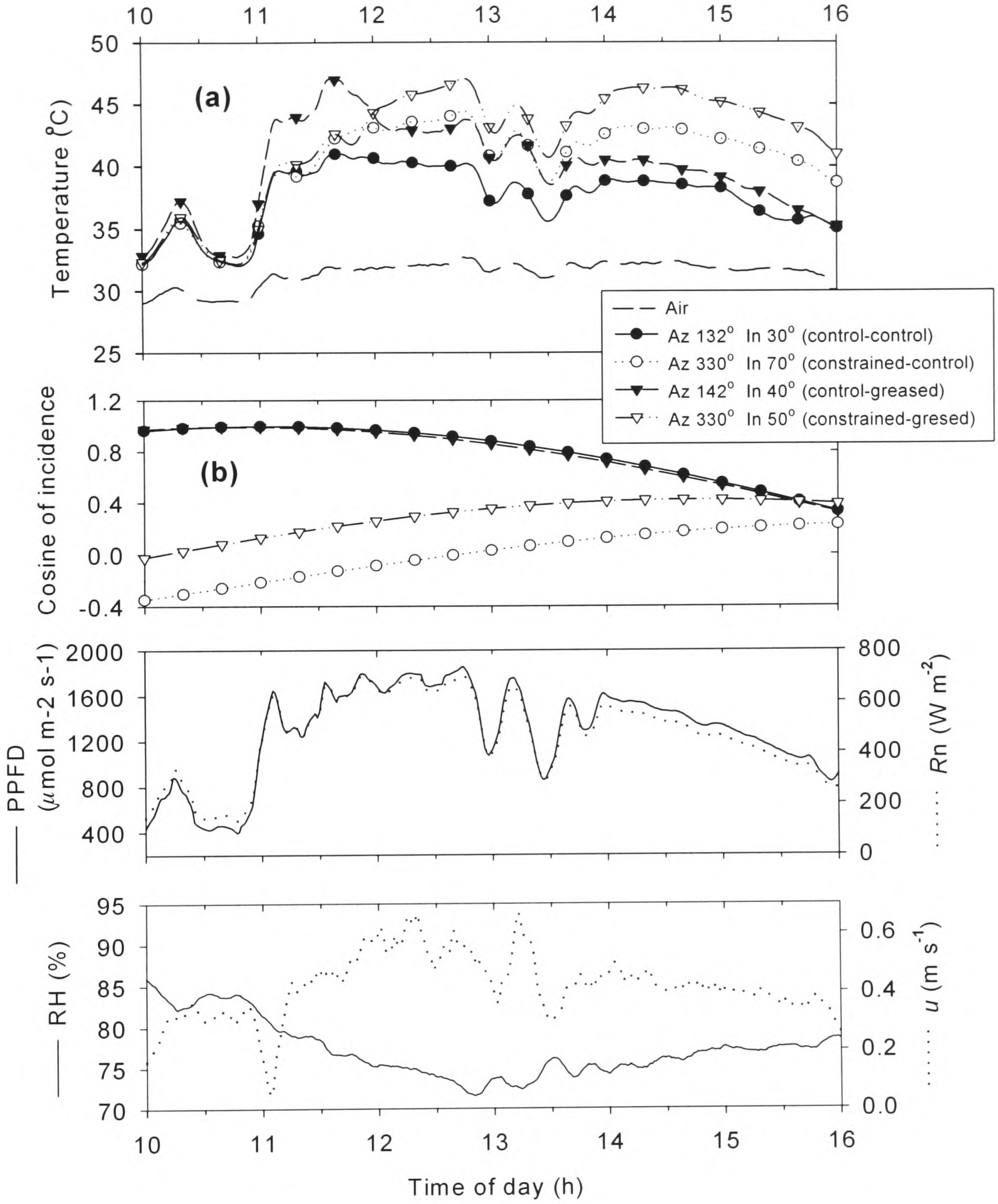


Figure 6.9 Gynoecium temperature, cosine of incidence and microclimate for flowers of *Merremia borneensis* under different treatments on the 23rd of January 1999. The treatments were a combination of constrained, transpiring and non-transpiring corolla and sepals.

gynoecium temperature and microclimate for *I. pes-caprae* were similar (data not shown for simplicity of presentation).

Changing position and restricting transpiration had a substantial effect on the temperature of the gynoecium of *M. borneensis* (Fig. 6.10). A flower turned 180° from its natural orientation caused and increase of temperature of about 4 °C relative to the naturally oriented flower. The excess temperature (the extent to which the tissue is warmer than the air) for a naturally oriented flower was around 8 K at around noon, and decreasing in the afternoon while the excess temperature for a turned flower was around 12 K and being constant during the day (Fig. 6.10a). The maximum excess (15 K) was reached by a non-transpiring flower naturally oriented at around midday (Fig. 6.10b). At this time of day the cosine of

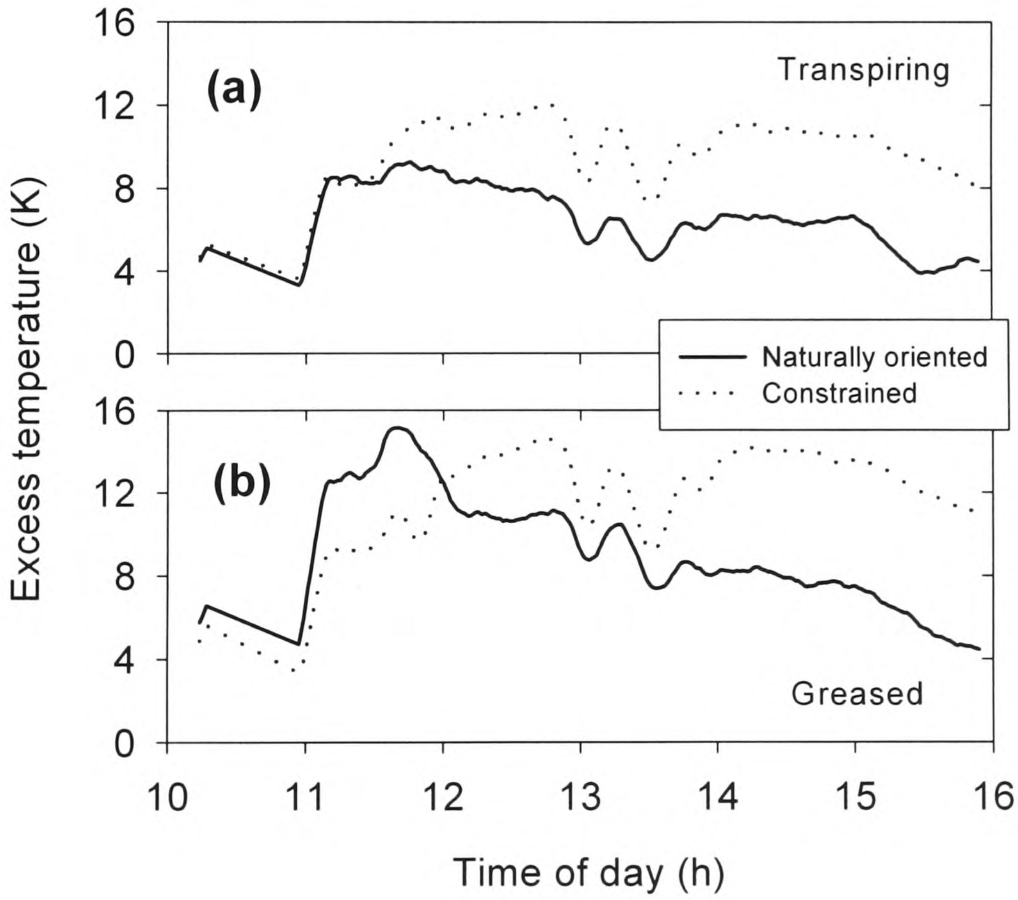


Figure 6.10 Excess temperature of the gynoecium of (a) normal transpiring flowers naturally oriented and constrained 180° from their natural orientation and (b) non-transpiring flowers naturally oriented and constrained 180° from their natural orientation.

incidence for the flower was almost 1.0 (Fig. 6.9b). After midday the excess temperature started to decline and this was related to the decrease in the cosine of incidence (Figs. 6.10b and 6.9b). The excess temperature for a constrained non-transpiring flower was about 14 K (Fig. 6.10b).

Further evidence for both, the relevance of transpiration as a cooling mechanism and position of the flower is described by the significantly different relationships between cosine of incidence and excess temperature of transpiring naturally oriented and constrained flowers (Fig. 6.11a) and non-transpiring naturally oriented

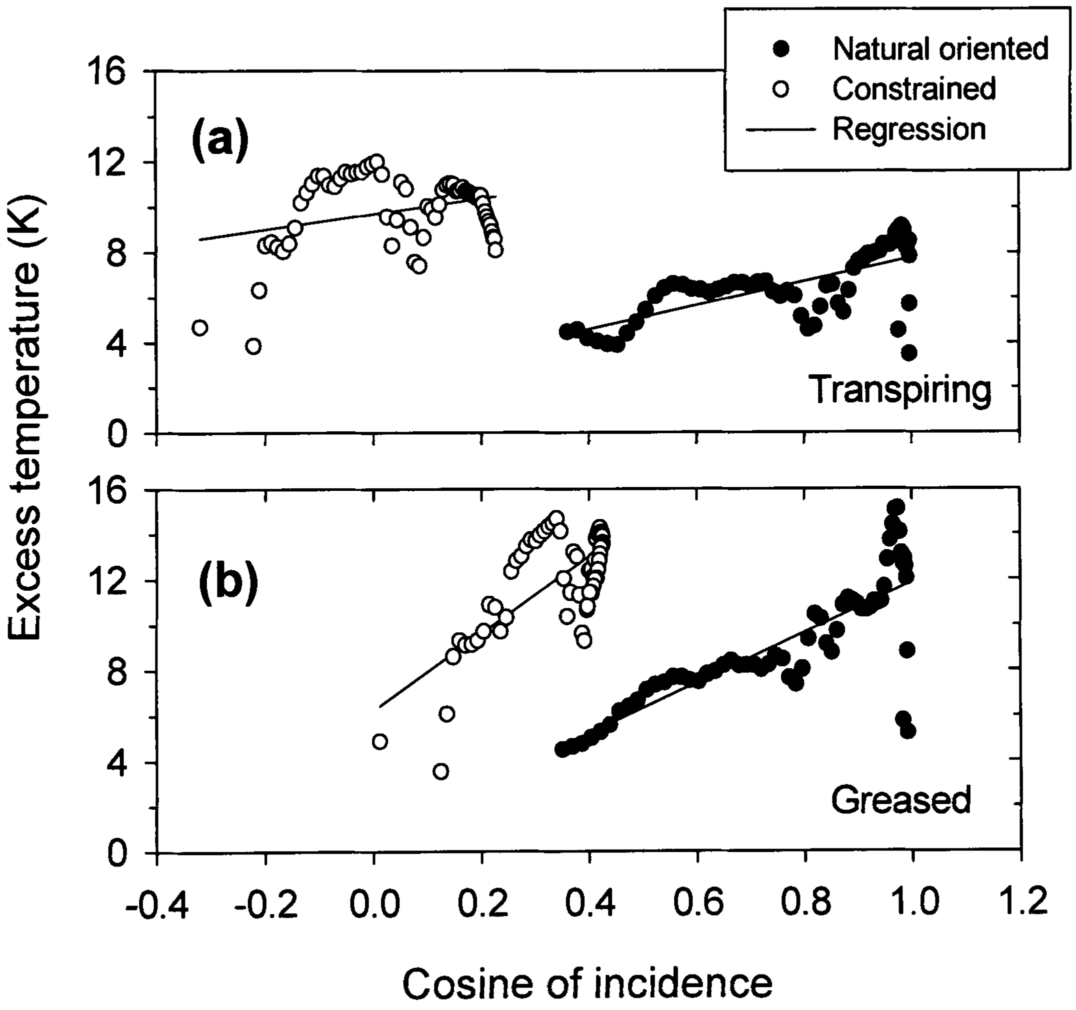


Figure 6.11 Correlation between the excess temperature of the gynoecium and the calculated cosine of incidence of sunlight for flowers of *M. borneensis* on the 23rd of January 1999. (**a**) Normal transpiring flowers and (**b**) flowers prevented from transpiration. The relationships were significantly different (Pearson's correlation coefficient) for transpiring naturally oriented (r=0.71, P>0.001) and constrained flowers (r=0.26, P<0.001) and for non-transpiring natural oriented (r=0.83, P<0.001) and constrained flowers (r=0.70, P>0.001).

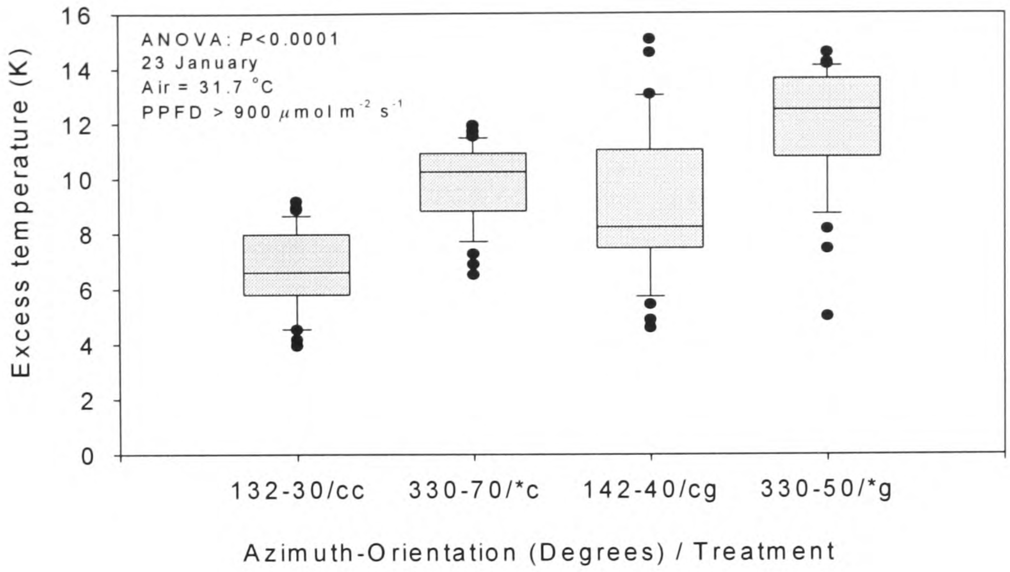


Figure 6.12 The excess temperature for *M. borneensis* treated flowers according to the position (azimuth-orientation) on the 23rd of January. Naturally oriented transpiring flowers (*cc*), constrained transpiring (**c*), naturally oriented non-transpiring (*cg*), and constrained non-transpiring (**g*). Mean excess temperature of each flower was used for comparison (One way Analysis of Variance) and they were significantly different.

and constrained flowers (Fig. 6.11b) of *M. borneensis* on the 23rd of January. Transpiration controls the gynoecium temperature even when the cosine of incidence is close to 1.0 (Fig. 6.11a *versus* Fig. 6.11b).

Figure 6.12 shows the mean values of excess temperature according to the orientation during the day. Naturally transpiring flowers normally oriented had the lowest mean values of excess temperature (6.7 K) and non-transpiring constrained flowers had the maximum mean value (12.8 K) again suggesting the cooling effect of transpiration and the shading effect of corollas on the temperature of the gynoecium

For *I. pes-caprae* on one experimental day on the 7th of September (Fig. 6.13), the minimum mean value (1.6 K) of excess temperature was obtained when flowers were normally transpiring and oriented to the west (270°) and the maximum mean value of excess temperature was obtained for non-transpiring flowers oriented east (7.3 K) when normally transpiring flowers were oriented east (90°).

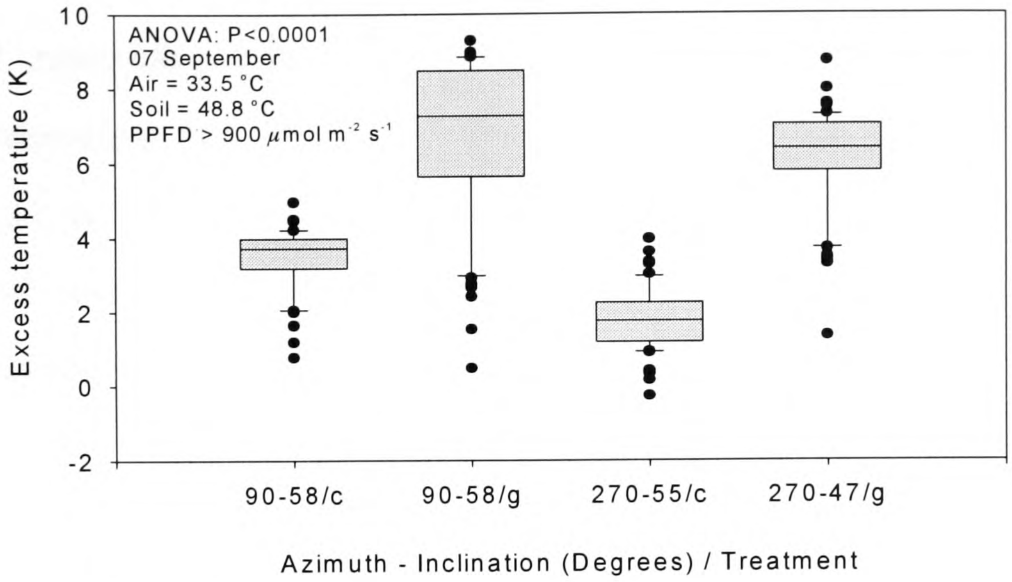


Figure 6.13 The excess temperature for treated flowers of *I. pes-caprae* according to the position (azimuth-orientation) on the7th of September 1998. Flowers oriented east normally transpiring (90°-55°/*c*), oriented east non-transpiring (90°-55°/*g*), oriented west transpiring (270°-55°/*c*), and oriented west non-transpiring (270°-47°/*g*). Mean excess temperature of each flower was used for comparison (One way Analysis of Variance) and they were significantly different.

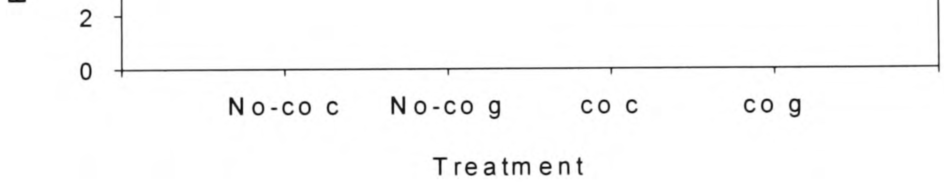


Figure 6.14 The excess temperature for treated flowers of *M. borneensis* on the 23rd of January 1999. Flowers with the corolla removed normally transpiring (No-co *c*), flowers with the corolla removed non-transpiring (No-co *g*), intact flowers transpiring (co *c*), and intact flowers non-transpiring (co *g*). Mean excess temperature of each flower was used for comparison (One way Analysis of Variance) and they were significantly different.

For *M. borneensis*, when comparing the gynoecium temperature of intact flowers and flowers with the corollas removed transpiring and non-transpiring (Fig. 6.14), the normal transpiring intact flowers had the minimum mean value of gynoecium excess temperature (6.7 K) while the non-transpiring flowers with the corollas removed had the maximum (11.4 K). The mean value of excess temperature for flowers with the corolla removed and normally transpiring was 8.2 K and for flowers with the corolla and non-transpiring the excess mean values was 8.3 K with more variance. The effect of removing the corollas from the flowers on the gynoecium temperature is shown in Figure 6.14. These results suggest that corollas are not only important for the pollination process but also contribute to maintain the gynoecium temperature at low levels.

Insect visitation

Of all the insects visited the flowers there were six main types from which it was possible to differentiate between pollen feeders (very small bees) and potential pollinators (small to big bumble bees, and wasps). Only two classes are considered here: pollen feeders and potential pollinators.The relationship between flower direction and frequency of flower visitors is shown for *M. borneensis* in Figure 6.15. The average number of visiting insects per flower was high for flowers with cosine of incidence close to 1.0 and low for flowers with cosine of incidence close to 0. The differences were significant ($P<0.04$ One-way Analysis of Variance, Minitab 12.3). These results indicate that insects prefer to visit flowers that are in the sun. When flowers were in the sun insects (potential pollinators) preferred intact flowers to flowers with the corolla removed and the differences were significant ($P<0.01$, Minitab 12.3; Fig. 6.16a). On different days, when the flowers were in the sun, insects seemed to prefer flowers with the corolla removed (Fig. 6.16b) this was due to the fact that pollen feeders were the main visitors of these flowers. For example on the 24th of January 76 % of the insects visiting flowers with the corolla removed were pollen feeders whereas 96 % of the insects

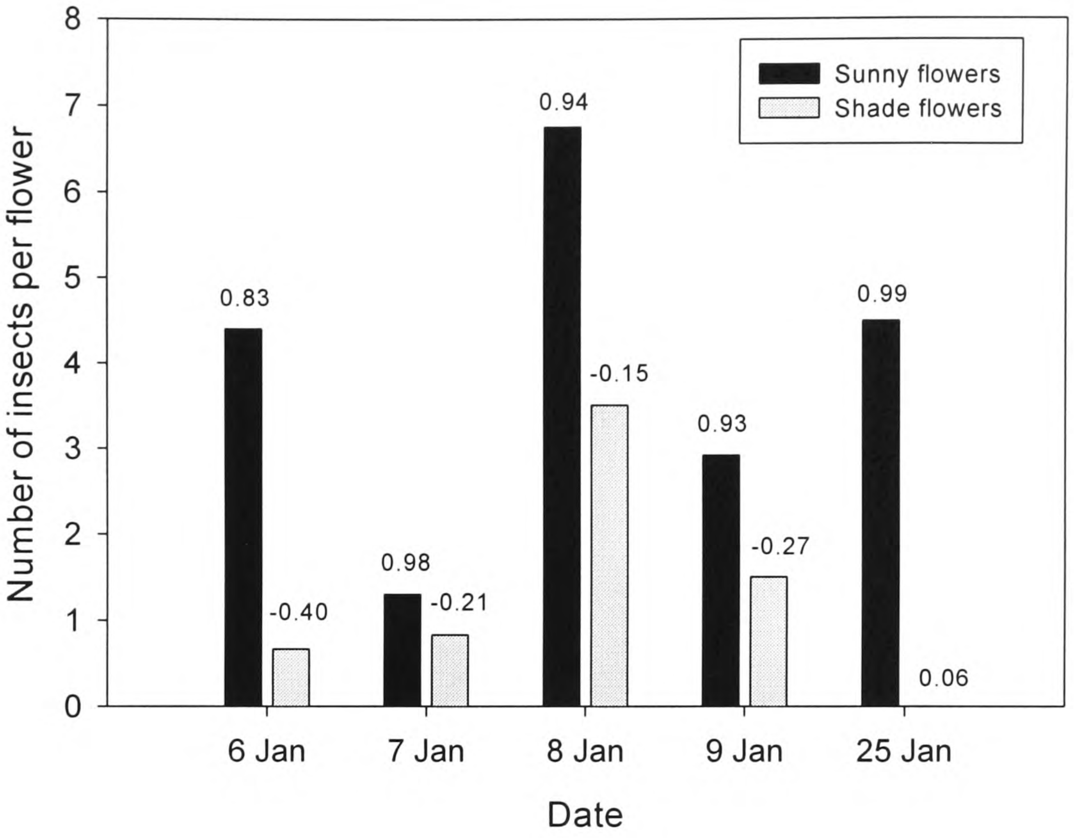


Figure 6.15 Mean number of flower visitors per flower in both conditions sunny and shade for different observation dates. The mean cosine of incidence is the number on top of each column. The differences between the conditions were significant (*P*<0.04, One-way Analysis of Variance).

visiting intact flowers were potential pollinators and 99 % of the insects visiting flowers with the stamens and pistils removed were potential pollinators. This result strongly suggests that potential pollinators of *M. borneensis* are attracted by the corollas in the sun. Further, some insects (one species was *Ameglia anomala*) visited the removed corollas that were lying in the ground. Few insects visited flowers in the shade (plants growing behind the bushes) either intact, with the corolla or the stamens and pistils removed (P<0.01, One-way Analysis of Variance, Minitab 12.3; Fig. 6.16c).A summary of relationship between flower orientation and frequency of insect visitors is presented for *I. pes-caprae* in (Table 6.5). The average of insect visitors per flower was higher for flowers facing west than for flowers facing east and the differences were significant (p<0.04, One-way

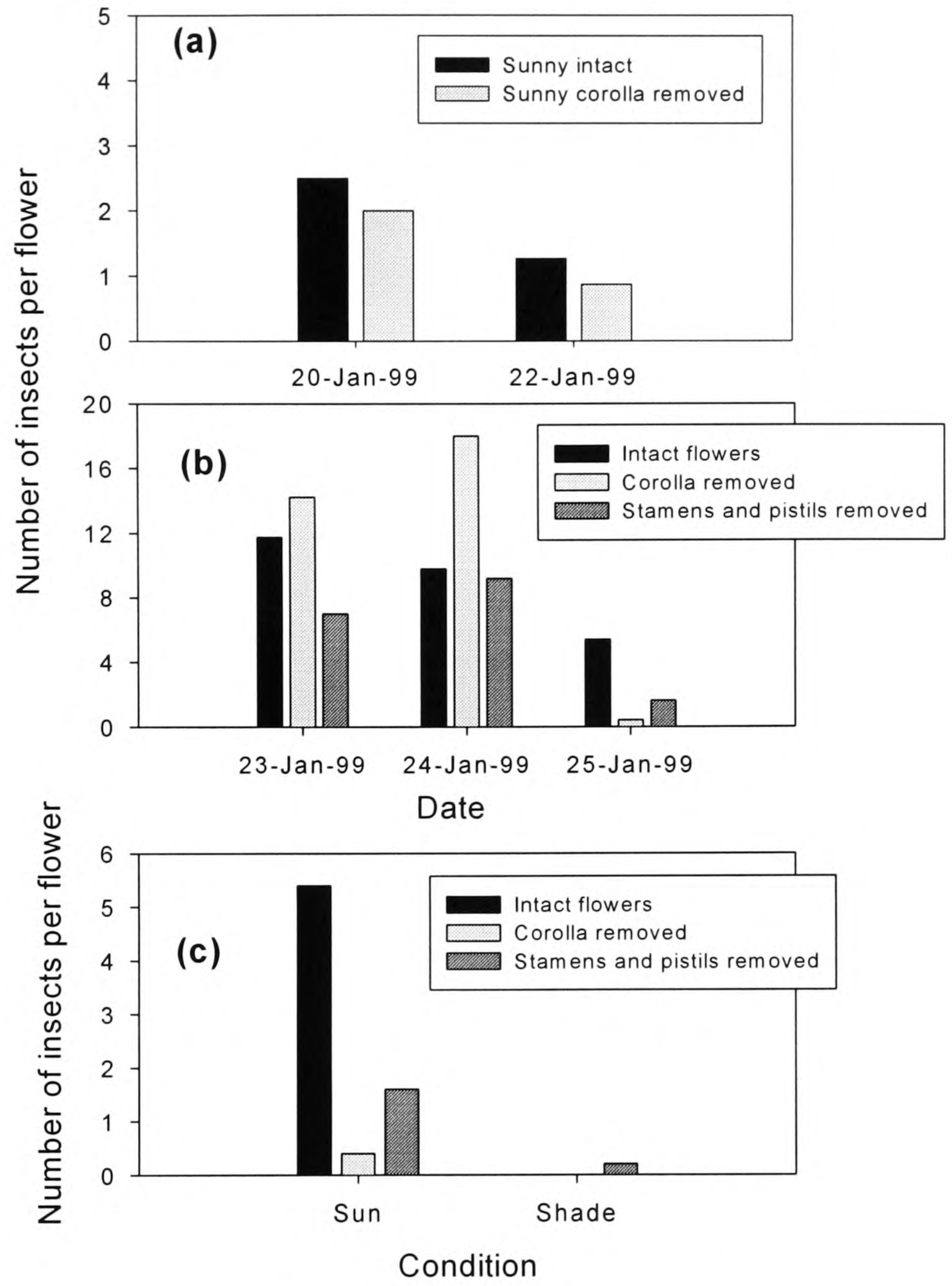


Figure 6. 16 Number of flower visitors per flower for *M. borneensis* under different conditions and treatments. (**a**) flowers with the corolla removed *versus* intact flowers in the sunshine; (**b**) flowers with the corollas removed, flowers with the stamens and pistils removed *versus* intact flowers in the sunshine; and (**c**) intact flowers, flowers with the corollas removed and flowers with the stamens and pistils removed in the sun *versus* the same treated flowers in the shade.

Analysis of Variance, Minitab 12.3). Note that the minimum mean value of excess temperature was obtained for normal transpiring flowers oriented to the west (Fig. 6.13).

Table 6.5 Mean number of flower visitors per flower in two direction classes (east = *E* and west = *W*) for *I. pes-caprae* in Meragang beach, Brunei for three days in September 1998. Observations were done in sunny days. Insects preferred to visit west facing flowers (P<0.04, one-way Analysis of Variance).

Date	Mean angle	Mean azimuth	Orientation	Number of insects	Number of flowers	Time observed	Insects per flower
07-Sep	44	64	E	35	25	60	1.4
07-Sep	45	301	W	29	8	60	3.625
Sep-02	45	120	E	0	5	60	0
Sep-02	45	320	W	74	5	60	14.8
Sep-13	45	270	W	130	8	45	16.25
Sep-13	45	140	E	0	5	45	0
Sep-13	45	90	E	38	8	45	4.75

6.4 Discussion

This chapter presents evidence for the non-random orientation of two tropical convolvulaceous flowers *M. borneensis* and *I. pes-caprae* and demonstrates that this adaptation enables them to lower the risk of overheating of the gynoecium and whilst ensuring pollination by insects.

Although these flowers appeared to face the sun during the hours of maximum solar radiation (morning to early afternoon) they do not show diurnal movements to follow the sun, and therefore they cannot be considered as solar trackers or heliotropic flowers. A leaf or a flower is considered to be a solar tracker or heliotropic when active movement occurs in response to the diurnal movement of the sun across the sky (Ehleringer and Forseth 1980) or "the diel bending or turning response, of plants directly to and with the sun" (Kevan 1972; Kevan 1975). According to Kevan (1972) "tropisms are exhibited through curvatures of sedentary plants".

On two occasions, flowers of *I. pes-caprae* were not significantly oriented in a mean direction. In these cases, the sites showed certain degree of disturbance; in the Coastal highway in Brunei (Fig. 6.5, Table 6.1), the flowers where growing in the parking lot in front of the University campus were people and cars disturbed the site. There was not a proper drain system, so when rain occurred the plants and consequently the flowers were covered with water and sand (personal observations). At the other site, a natural "beach" location (Sungai Liang) the measurements were carried out after the beach suffered disturbance due to a high

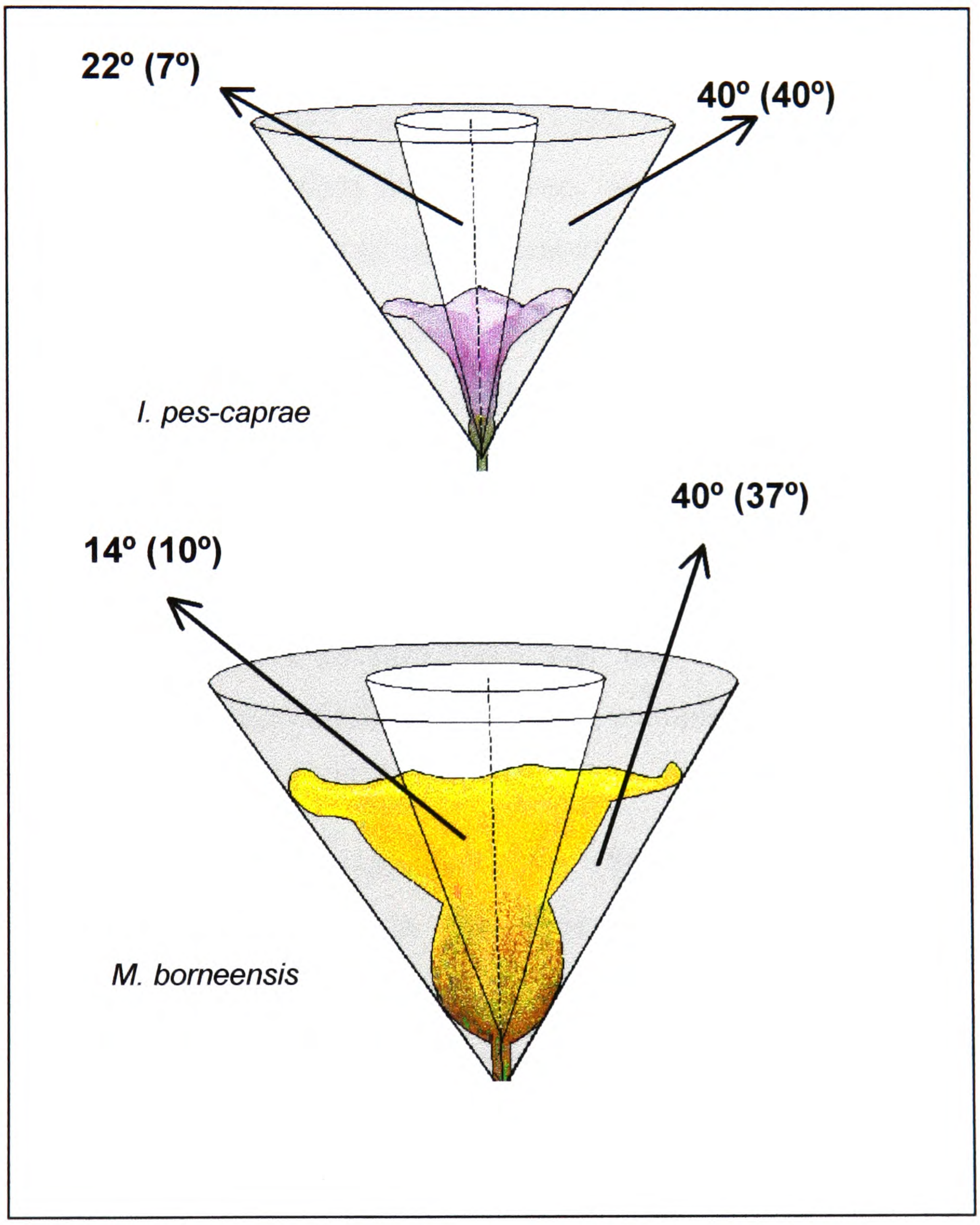


Figure 6.18 The parasol effect and critical angles. There is a critical range of angles within which the solar beam will not enter the corolla tube (and thus warm the gynoecium from the inside) and will not strike the lower region of the flower (and thus warm the gynoecium from the outside). This range is 22 – 40° for *I. pes-caprae* and 14 – 40° for *M. borneensis*. The angles calculated from photographs, corresponding to the critical angles, and depending on the exact shape of the corolla tube, are shown in parentheses.

tide, that shifted sand inundating plants so that flowers emerging from the sand could not have the normal orientation. This kind of disturbance may possibly be a normal part of the life cycle of this species because at that time plants were bearing mature fruits and the main agent for the dispersion of seeds is the seawater.

Apart from the two disturbed cases, there is evidence for a 'seasonal heliotropism' as flowers point towards the north or south parts of the sky, depending on the time of year. Although this response is evidently weak (much scatter is seen in Figs. 6.5 and 6.6), it is nevertheless distinct from Table 6.1. This appears to be the first record of seasonal heliotropism in the absence of diurnal heliotropism. Possibly different mechanisms are involved in this kind of heliotropism.

In contrast to flowers of the arctic and alpine species, the warming of the flower is unlikely to be a reward for the insect pollinators because the temperature of the air in these tropical lowlands is high enough to promote activity of pollinators. Then the question arises: why does a tropical flower faces the sun? There has been some discussion in the literature. For example flowers of *Drosera tracyi,* from the wet savannas in Florida, have inflorescences that face the morning sun. It was suggested that this pattern of heliotropism is an evolutionary anachronism because it apparently has no function (Wilson 1994).

The surface of the corolla is highly reflective (See Figure 6.17 in the appendix). Flowers with corollas facing the sun were more attractive to the potential pollinators than the corollas in the shade facing away from the sun. Pollinators discriminated among flowers on the basis of the position relative to the sun, and probably not because of the warm temperature on the flower chamber. However, facing the sun directly could be detrimental if the flower did not have mechanisms to cool down (Chapter 5). A notable difference between the tropical convolvulaceous flowers and all the known heliotropic flowers is that heliotropism is a mechanism to increase the temperature of the flower chamber, thus promoting pollination, fertilization, growth, and seed set.

As suggested by (Kevan 1989) and reported in chapter 5 corollas of some flowers with shapes different from a bowl shape provide a parasol for the gynoecium, but when the orientation of the flower is changed, for instance rotated 180°, the effect of the corolla as a parasol disappears.

Based on the shape of the flowers it was possible to calculate critical angles that are required to shade the gynoecium externally and to prevent direct irradiance into the centre of the trumpet (Fig. 6.18). If solar tracking were exact, the sun's rays would always enter the corolla tube and warm the gynoecium. However, by a certain angle away from the solar angle, this is prevented (Fig. 6.18). The cosine of incidence of the solar beam from all the flowers of *M. borneensis* varied from 0.77 (40°) to 0.97 (14°) and for *I. pes-caprae* it varied from 0.77 (40°) to 0.92 (22°). The trumpet shape of these flowers thus acts as a parasol shading the gynoecium at times of maximum solar radiation, and not allowing the rays to impinge on the gynoecium.

6.5 Apendix

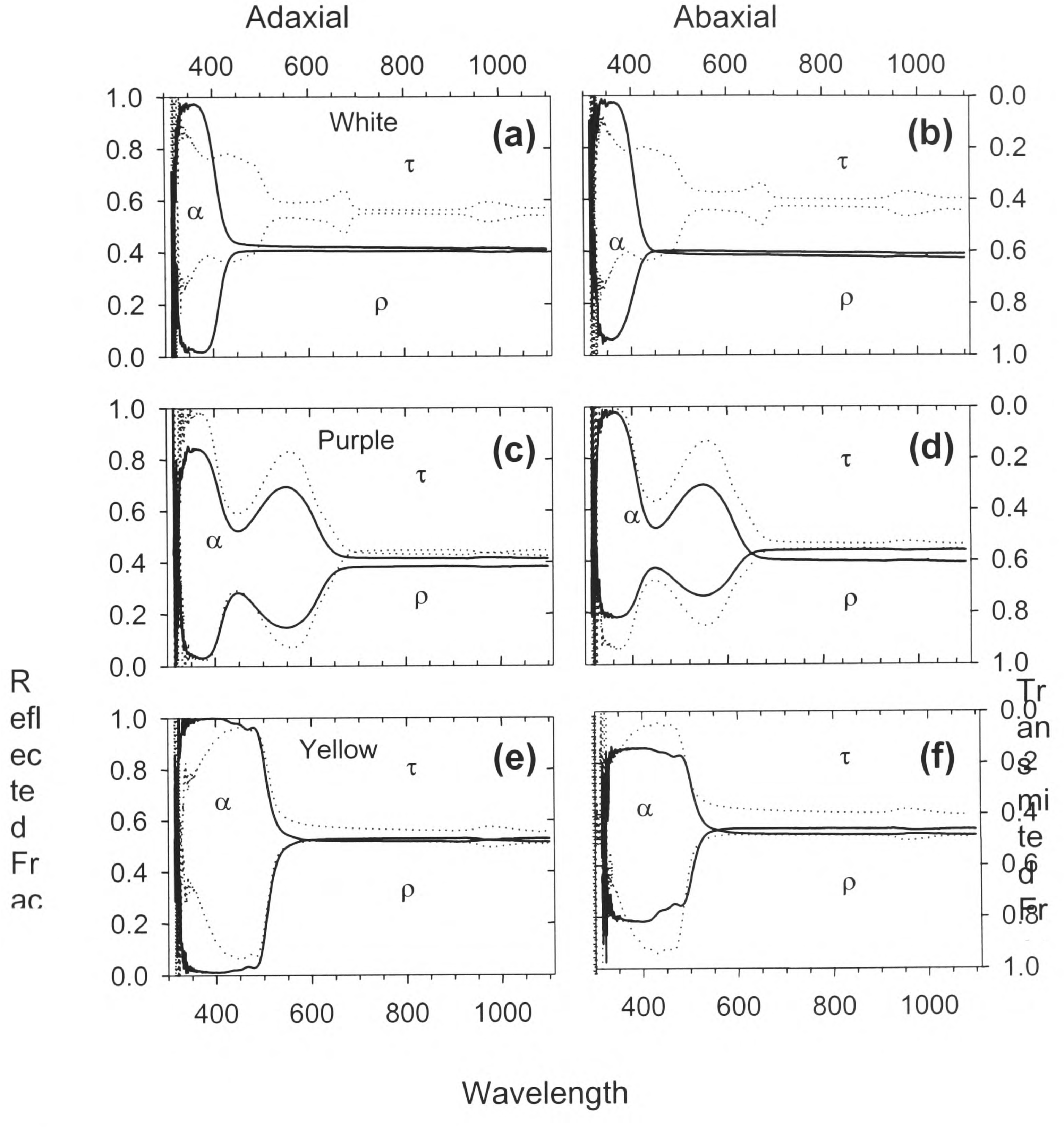


Figure 6.17 Spectral properties of petals. Representative fractions of irradiation absorbed (α), transmitted (τ), and reflected (ρ) by (**a**, **b**) white petals of *I. aquatica* and *Durio sp*; (**c**, **d**) Purple petals of *I. pes-caprae* and *Melastoma sp*; and (**e**, **f**) yellow petals of *M. borneensis* and *Dillinia sulfructicosa* as function of wavelenght (nm). Absorptances of the corollas to solar radiation in the 300-1100 nm waveband was measured on fresh pieces with a integrating sphere (LI-1800, LI-COR Inc., Lincoln, Nebraska). The flowers (10-15 per species) were collected in the morning and returned to the laboratory. Absorptance was measured for both abaxial and adaxial petal surfaces on April and December 1997. The petals of different species of flowers with similar colours to those of the convolvulaceous species used in this study were also investigated to check if the colour is an indicative of a defined radiative pattern. The species were *Dillinia sulfructicosa* (yellow), *Durio sp.*(white) and *Melastoma sp* (purple) all represented in the plots by the dotted lines.

6.6 Summary

1. Flowers of *Ipomoea pes-caprae* and *Merremia borneensis* show a preferred orientation, pointing in the general direction of the sun but not exactly tracking the sun.

2. The possible effects of this non-random orientation on the heat balance of the flower and the possible consequences on pollination were studied by measuring gynoecium temperature and insect visitation.

3. Differently treated flowers were used to measure gynoecia temperature along with the microclimate: intact flowers, flowers with corollas removed, flowers with the stile and stamens removed, shaded flowers, flowers constrained to be facing away from the sun. The lowest gynoecium temperature was achieved when the flowers were not constrained and not greased. It is concluded that the natural position of the flower, as well as transpiration, serves to reduce the temperature of the gynoecium.

4. Insects visited flowers in the natural position, and in the sun.

Chapter 7

General discussion

In a broad sense the aim of this thesis was to investigate some unexplored aspects of the functioning of flowers, focusing on the thermal aspects that relate to survival in a tropical environment.

In particular the work aimed to address the following questions:

(1) The flowers of the parasitic *Rafflesiaceae* occur in the dense shade of the forest understory. They attract pollinators by resembling rotting flesh and/or the wounds of animals, and producing volatile substances, a form of mimicry. Does the mimicry include the production of heat?

(2) The flowers of the *Convolvulaceae* are generally numerous, short lived, and produced in bright sunshine. They attract pollinators by being visually conspicuous but to achieve this they must face the sun. Do these flowers have a cooling mechanism, and if so, what is the nature of the mechanism?

(3) To what extent can flower temperature be said to be controlled?

The role of endothermy in flowers the of parasitic family Rafflesiaceae

In this thesis endothermy is reported for two species in the family *Rafflesiaceae*: *Rhizanthes lowii* and *Rafflesia tuan-mudae*. The question posed was: is endothermy part of the mimicry of the flower to attract the pollinating flies? Why would a flower from a warm tropical environment be endothermic?

Endothermy was detected in young buds of *Rhizanthes lowii* and mature buds of both species. Endothermy remained during blooming and *Rhizanthes lowii* showed signs of thermoregulation by maintaining fairly constant temperature during and after anthesis. *Rafflesia tuan-mudae* was shown to have only a weak pattern of endothermy. So, the machinery for endothermy is present at the early stages of floral development. The photosynthetic products which provide the respiratory substrate for endothermy are available 'free of charge' from the vine host. There would of course be a certain limit imposed by the productivity of the host. The vine may not have unlimited photosynthates for its parasites.

It has been demonstrated that many flowers increase their temperature above the ambient as a consequence of endothermy, the heat being produced by the cyanide-insensitive respiration (Meeuse and Raskin 1988; Skubatz, Williamson, Schneider et al. 1990). It may be that endothermy is a facultative characteristic in most plants and possibly all flowers. This assumption is supported by the fact that most plant mitochondria contain a cyanide and antimycin-insensitive alternative terminal oxidase (Lambers 1980; Moreau and Romani 1982). The alternative respiratory pathway has been detected in different tissues of many plants belonging to different taxa, for example ripening fruits of honeydew melons (Passam 1978), mango (Cruzhernandez and Gomezlim 1995), banana (Kumar and Sinha 1992) and avocado (Moreau and Romani 1982); roots and cotyledons of soybean (Day, Millar, Wiskich et al. 1994; Millar, Bergersen and Day 1994; Ribascarbo, Berry, Yakir et al. 1995); sugar beet callus (Shugaev, Vyskrebentseva and Shugaeva 1998); *Acer pseudoplatanus* (Aubert, Bligny, Day et al. 1997); rootstocks of pears (Tamura, Tanabe and Katayama 1996); root tuber of potato (Wagner, Vandenbergen and Krab 1992; Wagner, Vandenbergen and Wincencjusz 1995), sweet potato (Vandenbergen, Wagner, Krab et al. 1994), and cassava (Passam 1976); non-green tissues of *Petunia hybrida* (Wagner and Wagner 1995); in water stressed plants of sorghum (Kumar and Sinha 1994); shoot tips of Douglas fir (Fielder and Owens 1992); roots of white spruce (Weger and Guy 1991), *Convolvulus* (Van der Plas, Schoenmaker and Gerbrandy 1977), wheat (Lundegårdh 2001) and beans (Rychter, Chauveau, Bomsel et al. 1992).

As the tropical environment is rarely cold, it seems likely that endothermy in these parasitic plants is present as the result of the alternative respiration which is not coupled to energy conservation. High rates of respiration by the flowers are assumed to occur due to the nature of the flowers: non-photosynthetic, parasitic on the roots of the host where easily can obtain the substrate of respiration, then, endothermy may have evolved not merely to maintain the tissue at a high temperature (as in alpine species), but to ensure pollination. The suggestion from this present work is that the production of carbon dioxide, and possibly other volatiles is important. The flowers in this case are conspicuous by virtue of the carbon dioxide and/or volatiles they produce. The poorly mixed air at the forest floor may contain the olfactory signal to pollinators. A question to answer is whether or not CO_2 plays a role in the pollination of *Rhizanthes lowii* and *Rafflesia tuan-mudae*.

Because it was not possible to make direct measurements of gas-exchange in the field, a computational fluid dynamic model was employed[*] for estimation of the CO_2 concentration inside the diaphragm and in the reproductive cavity of the *Rafflesia* flower, based on the geometry and the measured wind velocity near the surface of the flower. The calculations suggest that the concentration of CO_2 in the reproductive cavity is about 60 times higher than the concentration of CO_2 in the understory of the forest.

Regarding the role of CO_2 as an insect attractant, it has been proved, for example, that CO_2 is the only attractant volatile of the larvae of western corn rootworm (*Diabrotica virgifera virgifera*, Coleoptera) to corn roots (Bernklau and Bjostad 1998). The detection of CO_2 by identified peripheral sensory organs of some terrestrial arthropods (e.g. nematodes, larva and adult beetles, centipedes, ants,

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[*] This work was made in collaboration with Dr. Tuula Aalto from the Physics Department of the University of Helsinki. Dr. Aalto modelled the heat exchange of the flower based on the geometry and wind speed measured at the surface of the flower. And by solving the Navier-Stokes equation which is the primary equation of computational fluid dynamics for an incompressible fluid with

termites, fig pollinators, honey bees, mosquitoes, flies, bugs, ticks, moths) is now well established, and the resulting coordinated behavioural responses at concentrations that occur naturally in the habitats of these organism has been well documented (Stange and Wong 1993; Stange 1996). Furthermore, there is evidence that the blowfly *Lucilia cuprina* has CO_2-specific sensory receptors (Stange 1975). It has been also demonstrated that CO_2 has an anaesthetic effect in blowflies and that they are considerably more sensitive to the CO_2 stimulus than to other anaesthetics (Diesendorf 1975). Thus, it may be possible that the CO_2 produced by the *Rafflesia* and *Rhizanthes* flowers play a role on the pollination by the blowflies.

The signals emitted by *Rafflesia tuan-mudae* and *Rhizanthes lowii* may be different. Despite *Rhizanthes* and *Rafflesia* attract blowflies of the same genera (*Lucilia*, *Chrysomya*, and *Hypopygiopsis* (Beaman, Decker and Beaman 1988; Bänziger 1991; Bänziger 1996; Hidayati, Meijer, Baskin et al. 2000), it has been observed that *Rhizanthes lowii* stimulated oviposition in the flies, suggesting that *Rhizanthes lowii* is releasing specific volatiles that trick female flies. Oviposition by the blowflies on *Rafflesia tuan-mudae* was not observed in this study and has not been observed on other *Rafflesia* species (Beaman et al. 1988; Bänziger 1991; Bänziger 1996). Detailed studies by Bänziger (1996) on the pollination ecology of *Rhizanthes zippelii* revealed that the pollination of this sapromyophilous flower is based mainly on brood-site deception, and only to a small extent on food reward (sugars produced at the nectar pads). The same pollination syndrome was observed in *Rhizanthes lowii* (personal observations). It seems that the genus *Rhizanthes* does not offer heat as a reward (because the air temperatures in the forest are sufficient for normal flight). Moreover, the food reward appears to be of secondary importance to the pollinator flies. This brood-site deception pollination syndrome has been described on blossoms of the South African desert stapelias

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constant viscosity, relating pressure and external forces acting on a fluid to the response of the fluid flow.

(Asclepiadaceae) (White and Sloane 1937; Meeuse 1961). Several *Stapelia* species have flowers that somehow (colour, shape, size) resemble the appearance of *Rhizanthes* and *Rafflesia* (Beaman et al. 1988).

Further work is required to see whether the olfactory signal is more complex than simply CO_2. Preliminary results from an ongoing study* on the identification of the emitted volatiles by *Rafflesia tuan-mudae* and *Rhizanthes lowii* revealed that *Rhizanthes lowii* emitted 3-Hydroxy-2-butanone, 2-Ethyl-1-hexanol and N,N-diethyl-3-methyl-benzamide compounds found in other flowers (Knudsen, Tollsten and Bergstrom 1993); and *Rafflesia tuan-mudae* emitted dimethyl disulphide and dimethyl trisulphide. These compounds have been found in various aroids flowers such as *Amorphophallus*, *Pseudodracontium* (Stransky and Valterova 1999), and *Hydrosme* (Kite and Hetterschieid 1997) *Hydnora* (Burger and Munro 1988) and in bat-pollinated flowers (Knudsen and Tollsten 1995) with dimethyl disulphide being the preferred component by the bats (von Helversen, Winkler and Bestmann 2000). Dimethyl disulphide and dimethyl trisulphide are compounds commonly related to bacterial growth on meet (Senter, Arnold and Chew 2000) and dimethyl disulphide alone is the compound that gives the taste to the Camembert cheese (Demarigny, Berger, Desmasures et al. 2000). It has not been possible to conclude this work in time for reporting in this thesis. Nevertheless, it is possible to formulate some hypothesis.

It may be possible that dimethyl disulphide and dimethyl trisulphide and perhaps other compounds (not identified), mixed with CO_2 have a synergistic effect in the attraction of the pollinating blowflies. It has been found that the antennae of the female blowfly *Lucilia cuprina* have a specific receptor neuron tuned to dimethyl disulphide (Park and Cork 1999) and it has been suggested that dimethyl trisulphide may be one of the major cues for calliphorid host finding (Nilssen, Tommeras,

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* The identification of the volatiles from the head-space of *Rafflesia* and *Rhizanthes* has been done in collaboration with Dr. Alice A. Edwards from the Chemistry Department of the University of Brunei,

Schmid et al. 1996). The location of the flower by the flies may follow the classical downwind model (Stange 1996) and once the flies have landed on the flower, mechanical and contact chemical inputs may guide the flies into the diaphragm and to the gynoecium. Although, *Rafflesia* is not a "trap" flower as defined by (Dafni 1984), it has been observed for *Rafflesia tuan-mudae* that few flies remain inside the diaphragm for some hours (personal observations), perhaps due to the $C0_2$ anaesthetic effect as suggested previously (Dafni 1984).

There may be some common characteristics among endothermic plants (Seymour and Schultze-Motel 1997), but *Rhizanthes* and *Rafflesia* seem not to comply. For example (1) most thermogenic flowers are monoecious and protogynous (the maturation of female flowers before those of male), while the flowers in this study were all unisexual; (2) endothermy is present only in ancient groups of flowering plants (Fig. 7.1). It is unclear whether the *Rafflesiaceae* are among the most primitive angiosperms as parasitism involves dramatic morphological and molecular changes (Schoen and Ashman 1995). It has not been settled whether it belongs in or near the Aristolochiales or whether it is a member of the Rosidae (Röse, Manukian, Heath et al. 1996). (Bremer, Chase and Stevens 1998) consider that it belongs neither in the phylogenetic basal orders nor in the monocots or the eudicots. In any case, it seems that endothermy in flowers is a homoplastic character present in different groups (Fig. 7.1); (3) thermogenic flowers are often pollinated by beetles (Seymour and Schultze-Motel 1997) and carrion flies (Uemura, Ohkawara and Kudo 1993). *Rafflesia* and *Rhizanthes* are pollinated exclusively by carrion or blowflies (Beaman et al. 1988; Bänziger 1991; Bänziger 1996; Hidayati et al. 2000). Thus, it seems that endothermy in flowers has evolved independently several times in different phylogenetic groups with different morphological organization and in each case it is associated with a particular pollination syndrome (Tang, Sternberg and Price 1987).

Brunei. I collected the samples in the field using the head-space technique and Dr. A. Edwards analysed the samples using a GC-MS.

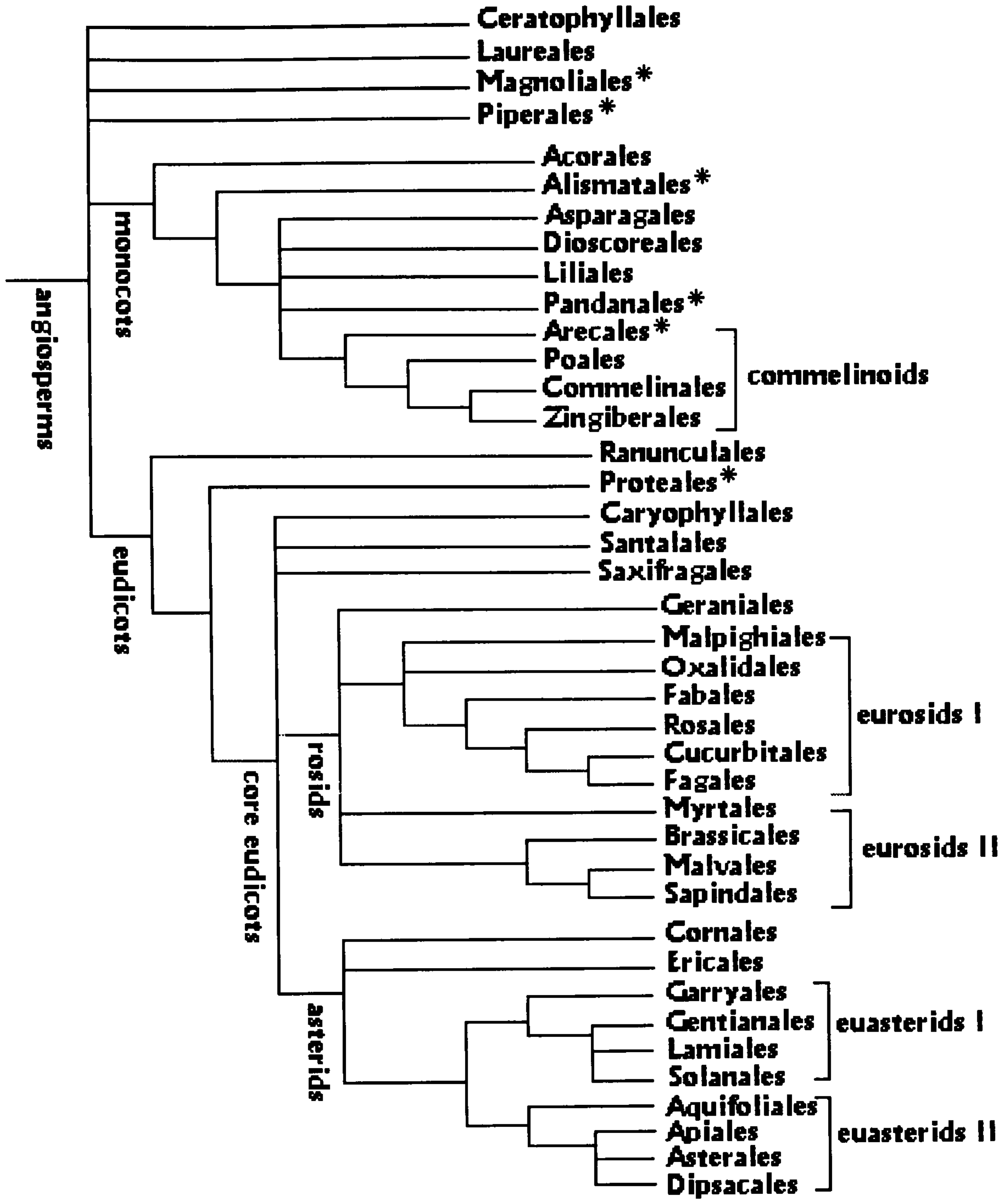

Figure 7.1 The position of the endothermic flowers within the angiosperm phylogeny group (modified from Bremer et al 1998). The cross indicates the groups containing endothermic families. 1) in the basal angiosperm groups: Magnoliales (Annonaceae and Magnoliaceae) and Piperales (Aristolochiaceae); 2) in the monocots: Alismatales (Araceae), Pandales (Cyclanthaceae), Arecales (Arecaceae); in the eudicots: Proteales (Nelumbonaceae). According to Bremer et al (1998) the Rafflesiacea neither belongs to any of the phylogenetic basal orders, not in the monocots or eudicots.

Further work is now needed on the behaviour of the pollinating flies, identification of the species of flies visiting the flowers.

A further step in elucidating the role of carbon dioxide in the pollination syndrome of *Rafflesia* and *Rhizanthes* would be to perform direct measurements of gas exchange, and careful identification of the volatiles that compose the odour, with bioassays of the substances using the pollinating flies. Experiments with model flowers, in which the odours are experimentally emitted, would enable identification of the role of specific gases as well as the importance of size, shape and colour of the flower.

Rhizanthes lowii showed signs of thermoregulation. It maintained fairly constant temperature at two days before anthesis, during anthesis and for the first two days of anthesis. Few flowers have been shown to thermoregulate (Nagy, Odell and Seymour 1972; Knutson 1974; Uemura et al. 1993; Seymour and Schultze-Motel 1996) and thermoregulation has been proposed as a reward to the insects that visit them by providing a warm and stable place to digest, grow and mate (Seymour and Schultze-Motel 1997). In *Rhizanthes lowii* it seems not to be the case, as *Rhizanthes* attracts the pollinating flies, which only visit the flowers and lay eggs on at mid morning and in the afternoon apparently with no reward to the insects. Why would the flowers spend energy in keeping a more or less constant temperature for as long as four days? It is possible that the flower does not have any specific mechanism to truly control temperature i.e. increase and decrease temperature at the particular times of day or moments in its life.

The cooling and orientation in convolvulaceous flowers

The convolvulaceous flower provides a sharp contrast with the parasitic *Rafflesiaceae*. The convolvulaceous flower seems to be an adaptation to life in habitats exposed to full sunlight. For example, the five species of *Calystegia* and *Convolvulus* occurring in Britain and Northern Europe are all found in such habitats: waste places, salt marshes, beaches, hedgerows and pathways (Table 7.1).

The present study on the tropical members of the family shows that the trumpet shape of the corolla of the convolvulaceous flower has a significant role in thermal budget of the flowers, whilst also playing the traditional role in the attraction of pollinators. For instance the corolla is orientated to act as a parasol shading the

Table 7.1 Species of convolvulaceous flowers in Britain and Northern Europe (Blamey and Grey-Wilson 1989).

Species	Common name	Habitat
Calystegia sepium	Larger Bindweed	Coastal salt marshes, beaches, sands and waste places
Calystegia soldanella	Sea Bindweed	Coastal dunes, sand and shingle
Calystegia sylvatica	Great Bindweed	Hedgerows, waste places
Calystegia pulchra	Hairy Binweed	Hedgerows, waste places
Convolvulus arvensis	Bindweed	Cultivated places, pathways, short turf, coastal

part of the gynoecium. The corolla also has the properties of cooling down the gynoecium whilst its strongly reflective properties provide the visual signal to attract pollinators. Flowers with trumpet shaped corollas may have been selected to survive in hot and sunny environments. The northern species (Table 7.1) may be "outliers", requiring warmth and so they may have different tropisms to the tropical Convolvulaceae. According to Kevan (1989), in alpine and temperate regions, natural selection favours flowers with shapes like flat plates or discs (*Asteraceae*) that absorb insolation according to the cosine of incidence of the sunlight, bowl shaped flowers (*Ranunculaceae*) that act as parabolic antennae focusing radiation into the gynoecium, inverted bells like *Saxifraga oppositifolia* which attain higher temperatures on the insolated side of the bell, or hanging bells like *Cassiope tetragona* that may gain heat by trapping warm air from the ground. Others have special arrangement, such as those that act as a micro-glasshouse like snowdrop with translucent floral parts and a massive pistil that absorbs shortwave radiation and re-radiates it at longer wavelengths inside the flower (Kevan 1989). All these shapes and arrangements favour the gain of heat, important in a cold climate, that promotes growth, rewards pollinators, and improves seed set (Kevan 1989).

Until now, there have been no comparative data on the thermal relationships or thermoregulation of temperate (late summer flowering species) or tropical flowers. They must maintain tolerable low temperatures while they are exposed to full sunlight. The temperatures near the soil in open places may exceed 40 °C, and the vapour pressure deficits under these conditions may be very high. Maintenance of turgor is required to retain the floral shape, and the scarcity of stomata on these petals is therefore not surprising. It is known for example that the temperate species *Helianthus annuus* (*Asteraceae*) reduces temperature by having diaheliotropic movements (Lang and Begg 1979).

I. pes-caprae and *M. borneenis* were seasonally heliotropic. So far, this phenomenon has not been reported. This characteristic may be present in other species in tropical locations. There are of course several exist some convolvulaceous species in northern latitudes, and would be interesting to compare the heliotropic mechanism in these species with those for the tropics.

Is there any reward to the pollinators? It seems clear that a heat reward is not necessary in the tropics. It is possible that the insects obtain nectar from the flowers. For example, large bumblebees that entered the trumpet of *M. borneensis* spent some time with their head at the bottom of the trumpet. The sepals of *M. borneensis* enclose large quantities of a viscous liquid (about 0.7 g per flower). It is likely that insects feed in this liquid (probably nectar).

The control of floral temperature

The idea of temperature control by flowers has long been suggested and demonstrated for alpine, andean and arctic flowers (Kevan 1972; McKee and Richards 1998). It is also known that pollination, fertilization, seed development are temperature sensitive processes sometimes inhibited by low temperatures and sometimes affected by high temperatures, and that temperature is one important factor in the limiting and distribution of plant species (Grace 1987). Flower size, shape, colour, position, orientation and the cellular structure of petal have been proposed as adaptations to increase floral temperature. Thermogenesis in flowers is another mechanism used by some flowers that helps to increase the floral temperature, ensuring pollination, fertilisation, floral development and seed set.

From this work it is possible to conclude that indeed there is a degree of temperature control in tropical flowers.

The parasitic understory plants produce their own heat, possibly to promote volatilisation of chemical compounds that attract the pollinators. High temperature in these flowers also may contribute to in the fertilisation process as it was found that endothermy persisted in both *Rhizanthes lowii* and *Rafflesia tuan-mudae* after the flowers had decayed.

The convolvulaceous flowers develop in a suitable position to avoid direct insolation into the gynoecium. The shape of the corolla plays the role of a transpiring parasol cooling the gynoecium. It is also an attractive corolla, enticing the pollinating insects.

In this thesis it is suggested that flowers are not only attractive devices to entice pollinators but also a physiological structure in which natural selection has acted in several ways to procure maintenance of the species.

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Oecologia (2000) 124:149–155

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Endothermy by flowers of *Rhizanthes lowii* (Rafflesiaceae)

Received: 29 August 1999 / Accepted 9 January 2000

Abstract *Rhizanthes lowii* (Beccari) Harms (Rafflesiaceae) is a parasitic plant that grows in the understory of the rainforest in South-East Asia. This plant does not have leaves, stems, or photosynthetic tissue and is characterised by the emission of a strong odour that attracts the natural pollinators, carrion flies. Flowers that volatilise odorous compounds and attract carrion flies, beetles and other insects are often thermogenic. Here we present evidence of both thermogenesis and thermoregulation in *R. lowii* from microclimate and tissue temperatures measured during different stages of flower development in *R. lowii*, in natural conditions in Brunei, Borneo. Endothermy was detected in young and mature buds as well as in blooming flowers and even in decaying tissues 3 or more days after blooming. Tissue temperatures were maintained at 7–9 K above air temperature, in both female and male flowers, at all stages of floral development.

Key words Thermogenesis · Thermoregulation · Mimicry · Parasitic plant · Pollination

Introduction

Endothermy in plants was discovered over 200 years ago (Lamark 1778) and has now been documented in nine different plant families: Cycadaceae (Skubatz et al. 1993), Nymphaeaceae (Prance and Arias 1975), Aristolochiaceae and Araceae (Knoll 1926; Knutson 1972; Meeuse 1966, 1975; Meeuse and Raskin 1988; Uemura et al. 1993), Arecaceae and Cyclanthaceae (Gottsberger 1990; Listabarth 1996; Schroeder 1978), Annonaceae (Gottsberger 1989, 1990), Magnoliaceae and Nelumbonaceae (Miyake 1898; Schneider.and Buchanan 1980).

Several hypotheses have been presented for the ecological relevance of elevated temperatures in plants (Moodie 1976; Uemura et al. 1993): to protect the flowers from freezing (Camazine and Niklas 1984; Knutson 1974), to enhance the rate of flower development (Bermadinger-Stabentheiner and Stabentheiner 1995), to provide a direct energy reward for insect pollinators (Cooley 1995; Kevan 1975; Prance and Arias 1975; Seymour and Schultze-Motel 1997; Seymour et al. 1983), to increase diffusion rates of carbon dioxide and volatilisation of specific chemicals that attract pollinators (Bahr and Bonner 1973; Chen and Meeuse 1971; Meeuse 1966, 1975; Moodie 1976; Nagy et al. 1972; Schneider and Buchanan 1980; Skubatz et al. 1990; Smith and Meeuse 1966; Uemura et al. 1993), to enhance the growth of the pollen tube (Jewell et al. 1994; Kudo 1995), to assure reproductive success (seed set) (Miller 1986) and to achieve mimicry of mammalian faeces and carrion to attract scavengers and carrion flies (Knutson 1972; Moodie 1976; Uemura et al. 1993).

A few species of plants have flowers that go beyond endothermy and actually regulate their temperature: *Philodendron selloum* (Nagy et al. 1972), *Xanthosoma robustum* (Meeuse and Raskin 1988), *Symplocarpus foetidus* (Knutson 1974), *S. renifolius* (Uemura et al. 1993) and *Nelumbo nucifera* (Seymour and Schultze-Motel 1996). They maintain a nearly constant internal temperature despite large fluctuations in air temperature (Seymour 1997).

The genus *Rhizanthes* belongs to the wholly parasitic family Rafflesiaceae. It contains only two species: *Rhizanthes zippelii* (Blume) Spach and *R. lowii* (Beccari) Harms distributed in Borneo, Sumatra, Malaysia, Java, and Thailand (Bänziger 1995; Meijer 1997).

Rhizanthes is parasitic on the roots and near-ground stems of a few species of the vine *Tetrastigma* (Vitaceae), and is therfore an understory plant. It has no leaves

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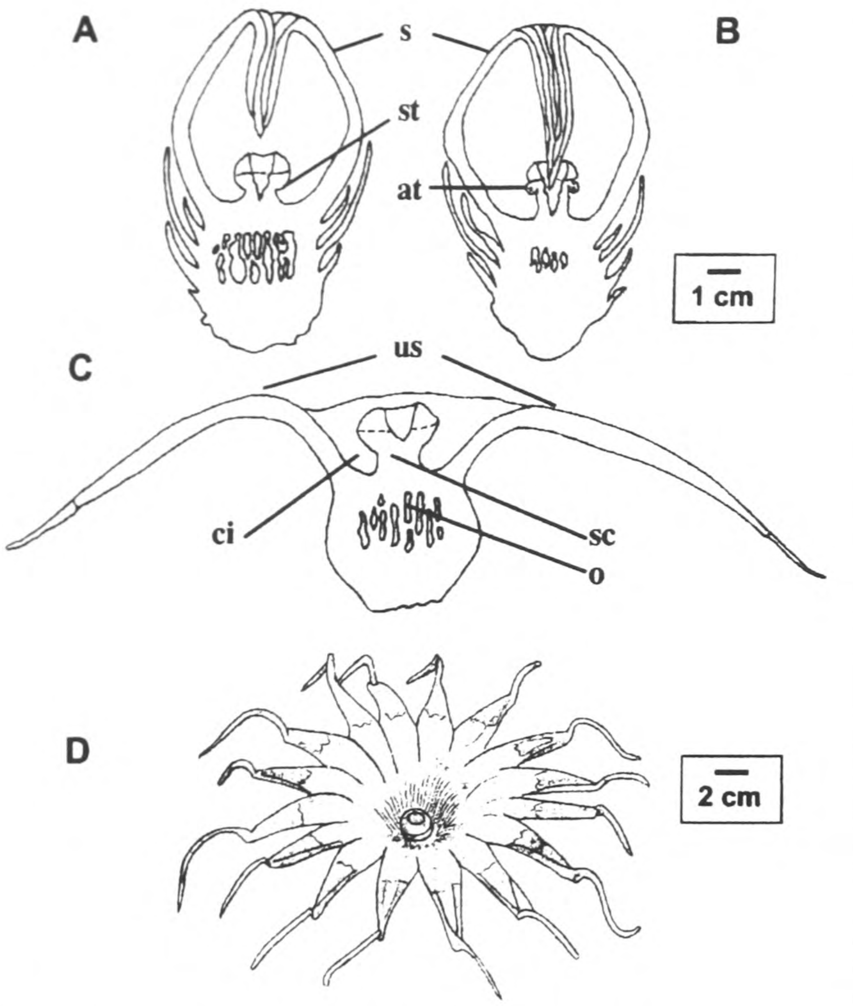

Fig. 1 Schematic representation of a median longitudinal section through a female bud (**A**), male bud (**B**) and open female flower (**C**) showing the different parts; and *Rhizanthes lowii* in bloom (**D**) [*s* bud surface, *st* stigmatic fascia, *at* anther, *us* abaxial surface of the petal, *ci* circumambulator, *sc* stalk of the column, *o* ovary (female), interior (male)]

or photosynthetic tissue, stems or roots, the only vegetative parts being fine filaments that penetrate the roots and stems of the vine host. The plant produces large ephemeral flowers that are unisexual in *R. lowii* and unisexual or bisexual in *R. zippelii*.

The plant is first evident as a small protuberance emerging from the roots of the host. After 6–7 months, it takes the form of a brownish bud (Fig. 1A,B) which blooms into a large flower (Fig. 1D). This attracts insects of several families by producing a strong "stuffy room" and "cheese-like" odour (Bänziger 1996), or a "cadaverous" smell (some authors in Meijer and Veldkamp 1988). Of the many insect visitors, only female carrion flies of the genera *Lucilia*, *Chrysomya* and *Hypopygiopsis* effect pollination (Bänziger 1996). Bänziger suggested that the hairyness of *R. zippelii* mimics mammalian pelage whereby the reproductive parts resemble a carcass, or mimics orifices or wounds of a hairy mammal, whilst producing the various volatile essences and probably CO_2 that mimic the odour of carrion, wounds or infected orifices. These stimuli induce mistaken oviposition, the hatchlings being doomed to starvation.

In the present paper we test the hypothesis that *R. lowii* is an endothermic plant, as suggested by its appearance, mode of life and pollination biology.

Materials and methods

Study site

This study was conducted in the Batu Apoi Forest Reserve at the Universiti Brunei Darussalam Kuala Belalong Field Studies Centre (KBFSC; 115°8′ E, 4°32′ N), Brunei, Borneo, S.-E. Asia. The forest at KBFSC is classified as a pristine mixed dipterocarp forest (20–800 m above sea level), with clay and sandstone humus-rich soils. The climate is aseasonal, with mean monthly rainfall >100 mm for all months. The site included a small valley and the adjacent north-facing slopes. Ashton (1964) reported detailed descriptions of vegetation, climate and soils.

Plant material

R. lowii flowers are rare and found in a very restricted habitat (evergreen, primary tropical rainforest, 120–160 m above sea level). Six clusters of *R. lowii* with 9–15 buds at all stages were distributed over an area of about 5 ha. All the clusters were parasitic on *Tetrastigma pedunculare* (Wall. ex Laws.) Planch. (Vitaceae). Individual buds were tagged to follow growth and survival. In this study, flowers that were blooming were of the same sex in each cluster. Some buds from different clusters were selected (usually the mature ones) to follow the pattern of temperature during the development of the bud until blooming and decay.

Four flower stages were classified as (1) young bud, defined as several weeks to 1 week before anthesis, (2) mature bud, at 7 days prior to anthesis, (3) fresh flower, the 1st and 2nd days following anthesis and (4) decaying flower, from the 3rd day following anthesis.

Field measurements: flower temperature and microclimate

The diurnal pattern of temperature was measured on two female and two male flowers of *R. lowii* during the natural sequence of anthesis (before, during and after blooming), and four immature buds from different clusters. Measurements were made during three periods from 16 October to 14 December 1997. Copper-constantan (0.5 mm in diameter) thermocouples were inserted in different parts of the buds to measure the temperature of: bud centre (i) and bud surface (s) (Fig. 1A,B); and in the open flower: ovary (female), interior (male) (o), anther (male) (at), stigmatic fascia (female) (st), stalk of the column (sc) and upper surface (us) (Fig. 1C). Soil temperature (so) was also recorded with an additional thermocouple 5 mm below the soil surface. Air temperature, (T_a), and relative humidity (RH) were measured with thermocouples in a custom-built forced-air hygrometer. Wind velocity, *u*, at flower level at about 10 cm from the ground was measured with an omnidirectional hot-wire anemometer (model 8460-13E-V; TSI, St. Paul, Minn.). Wind velocity at 30 cm above the ground was measured with a cup anemometer (Model MG2; Vector Instruments.). Net radiation, R_n, was measured with a Funk-type net radiometer (Q-7 Net Radiometer; Campbell Scientific, Leicestershire, UK). The net radiometer was placed 10 cm above the ground near the bud or open flower. Photosynthetic photon flux density (PPFD) was recorded with a quantum sensor placed just above the subject (Quantum Sensor SKP215; Sky Instruments, Llandrindod Wells, UK) (data for wind and PPFD are included in Fig. 3).

All variables were measured every 6 s and recorded as 1-min means during the day, and every 20 s with 5-min means during the night. Data were stored automatically on a data logger (Model 21X; Campbell Scientific). Later, they were plotted as average 10-min values. To avoid any temperature effect of sunflecks on the flowers, one of the female flowers was covered with a sheet of white corrugated plastic placed 1.0 m above the ground.

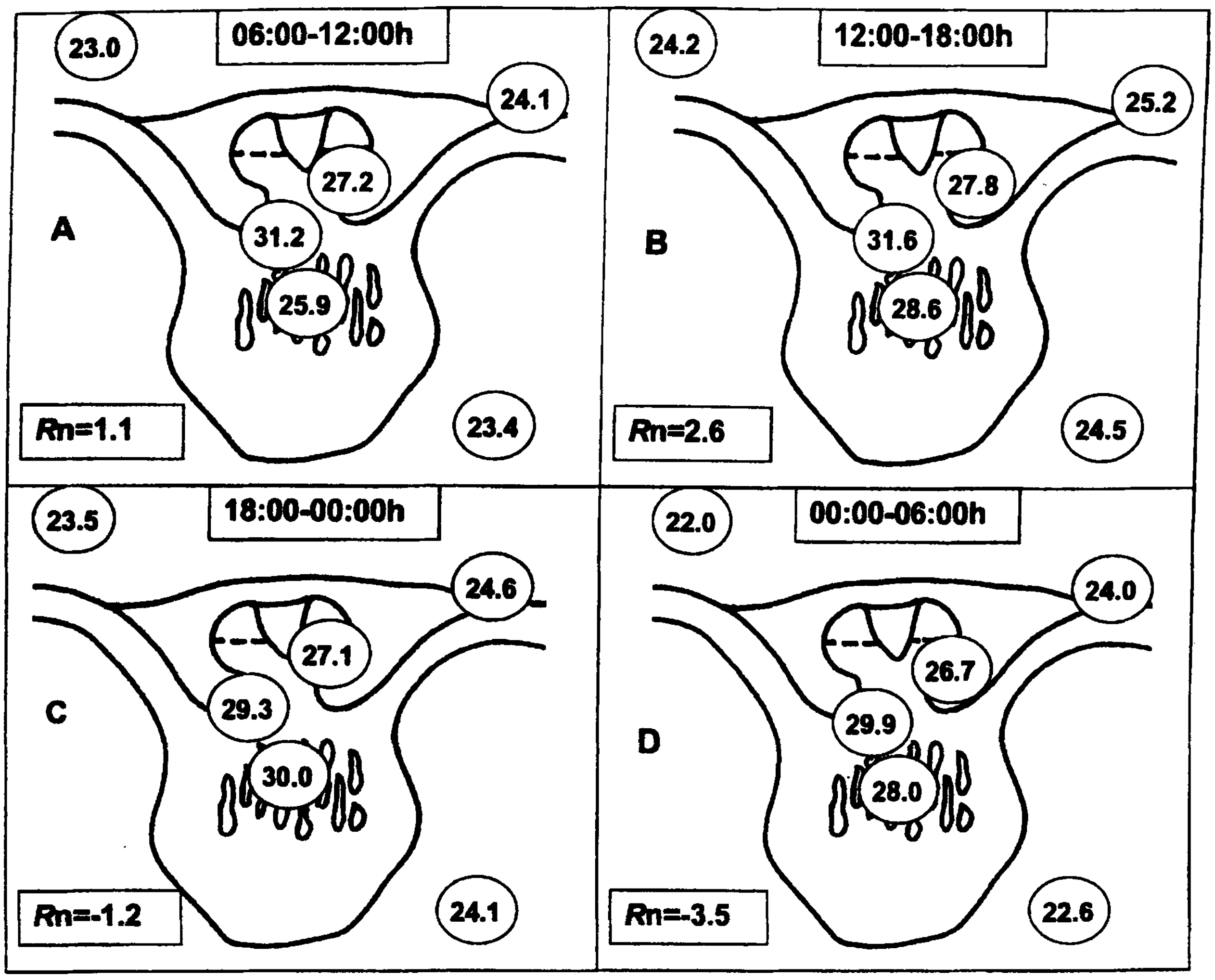

Fig. 2A–D Schematic representation of tissue temperature for a female flower during the day. The day is divided into four periods of 6 h each. Each temperature value represents the period mean temperature over 2 days for a fresh flower. The *values inside ovals* are the mean temperatures in °C. Net radiation (R_n) is given in W m^{-2}

Results

Diurnal changes in temperature were studied for several days on two male and two female flowers, and four young buds. The patterns presented in this set of graphs were similar for the four blooming flowers of this study regardless of the sex, but only one is shown for simplicity of presentation. Some differences between flowers will be indicated in the text.

The mean temperature of different parts of the fresh flower over 2 days is shown in Fig. 2, grouping data according to time of day. All tissue temperatures were consistently warmer than the air, often by more than 6 K in the centre of the floral structure. The net radiation was always low, in the range +2.6 to –3.5 W m^{-2}. The stalk of the column (the structure that limits the region visited by pollinating insects; see Fig. 1C) did not vary much, being 30±1°C, whilst the ambient temperature varied around 23±1°C and the soil varied about 22.5±2°C. The ovary was predominantly warm during the night (Fig. 2C) and the stigmatic fascia was almost constant for the four periods. The surface temperature fluctuated from 25° 28°C and followed the diurnal pattern of ambient temperature.

The term "excess temperature" will hereafter be used to indicate the extent to which the tissue is warmer than the air and will be expressed in K.

Figure 3A–E shows a representative 10-day course of excess temperature and microclimatic variables for a young bud and a female flower in November 1997.

One young bud and one mature bud were studied simultaneously for almost 10 days until premature abscission of the young bud on day 7 (Fig. 3A). The mature bud opened into a flower in the early morning of day 7 (Fig. 3B). The interior of the young bud (line 1, Fig. 3A) was 1 K above T_a at the beginning of the study period and 4 K before abscission whilst the surface (line 4, Fig. 3A) never exceeded 1 K. The excess temperature of the internal tissue increased every day in the late afternoon, when rain usually fell. The diameter of the bud increased at a rate of about 3 mm per day (from 25 to 40 mm). Figure 3B shows the diameter of the mature female bud which was about 60 mm at 6 days before anthesis, increasing by approximately 3 mm per day, and reaching 75 mm on the day before anthesis (day 6, Fig. 3B). For male buds, the increment in diameter was 1.3 mm per day reaching 69.5 mm at the moment of anthesis (data not shown).

During the period of measurements, as the bud increased in size, the excess temperature of the stalk of the column rose over a 5-day period (days 2–6; Fig. 3B) with a tendency for maxima to occur in the late afternoon and early in the morning prior to anthesis. The surface temperature of the mature bud (line 4, Fig. 3B) remained fairly constant at about 3 K above the air during the 6-day period prior to anthesis, increasing on the night of anthesis (day 7; Fig. 3B) to the maximum excess temperature of 4.1 K. This suggest a high rate of metabolic activity during anthesis. The surface temperature of the flower (day 7–10; Fig. 3B) was close to air temperature with a maximum excess of about 1.5 K.

The ovary temperature (line 1, Fig. 3B) was measured from the day of anthesis, showing its maximum excess temperature of 7.8 K on the first night of blooming, en-

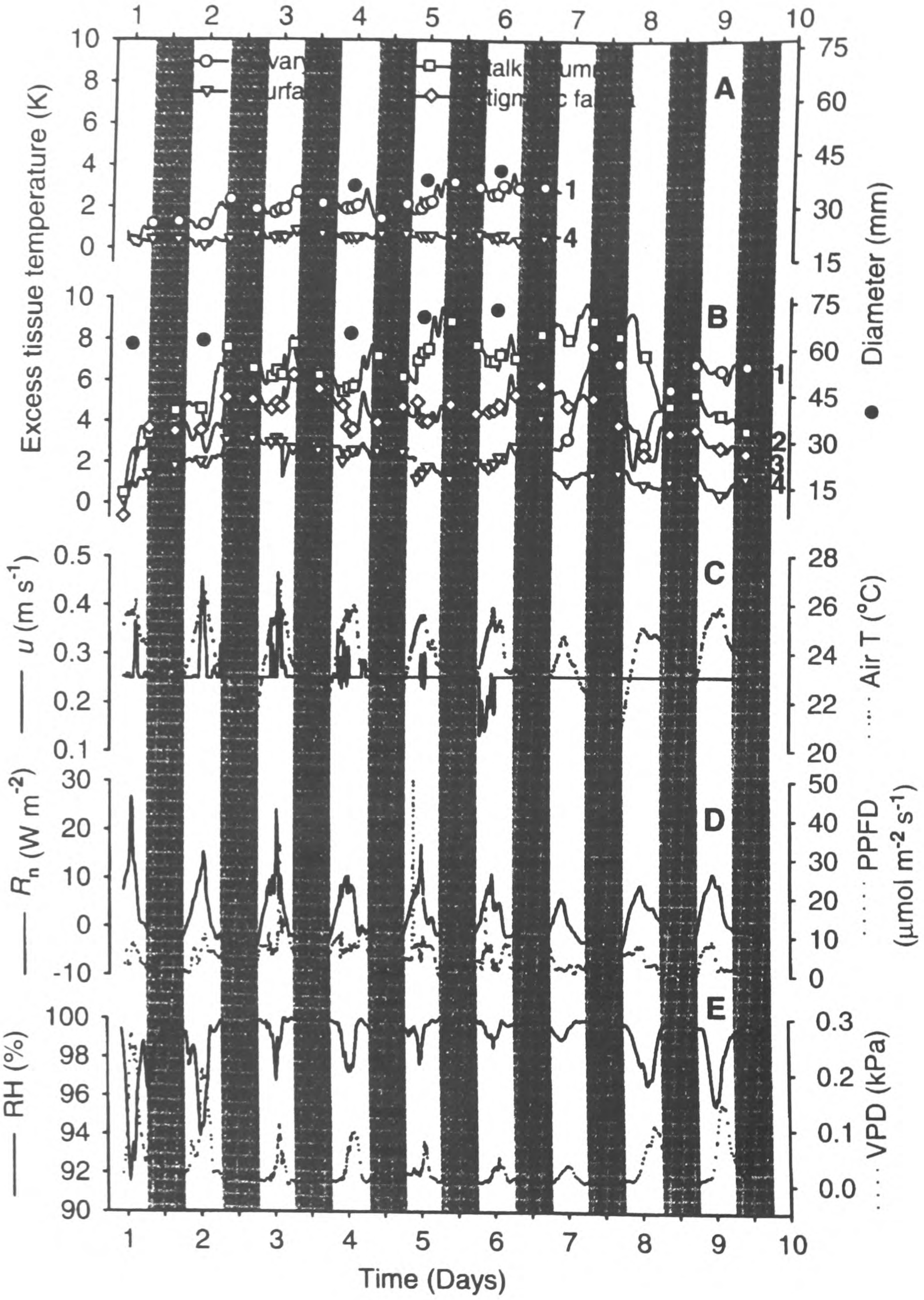

Fig. 3A–E Excess temperature and the microclimate of bud and female flower parts of *R. lowii* from the 17 to 26 November 1997. Nights are indicated by *grey shadows*, *closed circles* indicate bud diameter, the *arrow* indicates anthesis. **A** Young bud: interior (*line 1, circles*) and surface (*line 4, triangles*). **B** Excess temperature courses for flower parts: ovary (*line 1, circles*), stalk of the column (*line 2, squares*), stigmatic fascia (*line 3, diamonds*) and surface (*line 4, triangles*). The figure follows the sequence of anthesis: mature bud (day 1–6), anthesis (*arrow*), fresh flower (day 7–8) and decaying flower (day 9–10). **C** Wind velocity (*u*; *solid line*) and air temperature (*dotted line*). **D** Net radiation (R_n; *continuous line*) and photosynthetic photon flux density (PPFD; *dotted line*). **E** Relative humidity (RH; *solid line*) and flower surface-to-air vapour pressure deficit (VPD; *dotted line*)

tering a cooler period on the 2nd day, and reaching a minimum excess of 1.6 K, increasing again on the 2nd night to 7.2 K. Thereafter, it was maintained fairly constant at 6.5 K during the decaying period.

The maximum excess temperature was as high as 9.6 K (day 7; Fig. 3B) and was achieved in the stalk of the column on the first day of blooming (line 2, Fig. 3B). Three peaks of excess were observed in the stalk of the column during the fresh flower stage: one in the morning, one in the afternoon on the 1st day and one in the morning on the 2nd day. These peaks were coincident with the time of maximum activity of flies (10–11 h and 17–18 h; personal observation).

The excess of the stigmatic fascia (day 3–6; line 3, Fig. 3B) was particularly constant during the mature bud stage, reaching a maximum excess of 6 K and started to decline after the 1st day of anthesis.

The average net radiation during the day was about 8 W m^{-2} and never exceed 30 W m^{-2}; PPFD was normally 13 µmol m^{-2} s^{-1} during the day, with a few sunflecks at midday not exceeding 50 µmol m^{-2} s^{-1} (Fig. 3D). Wind velocity at the flower level never exceeded 0.5 m s^{-1} and air temperature fluctuated between 21°C and 26°C (Fig. 3C). The relative humidity was very high and vapour pressure deficit was extremely low during the whole period (Fig. 3E).

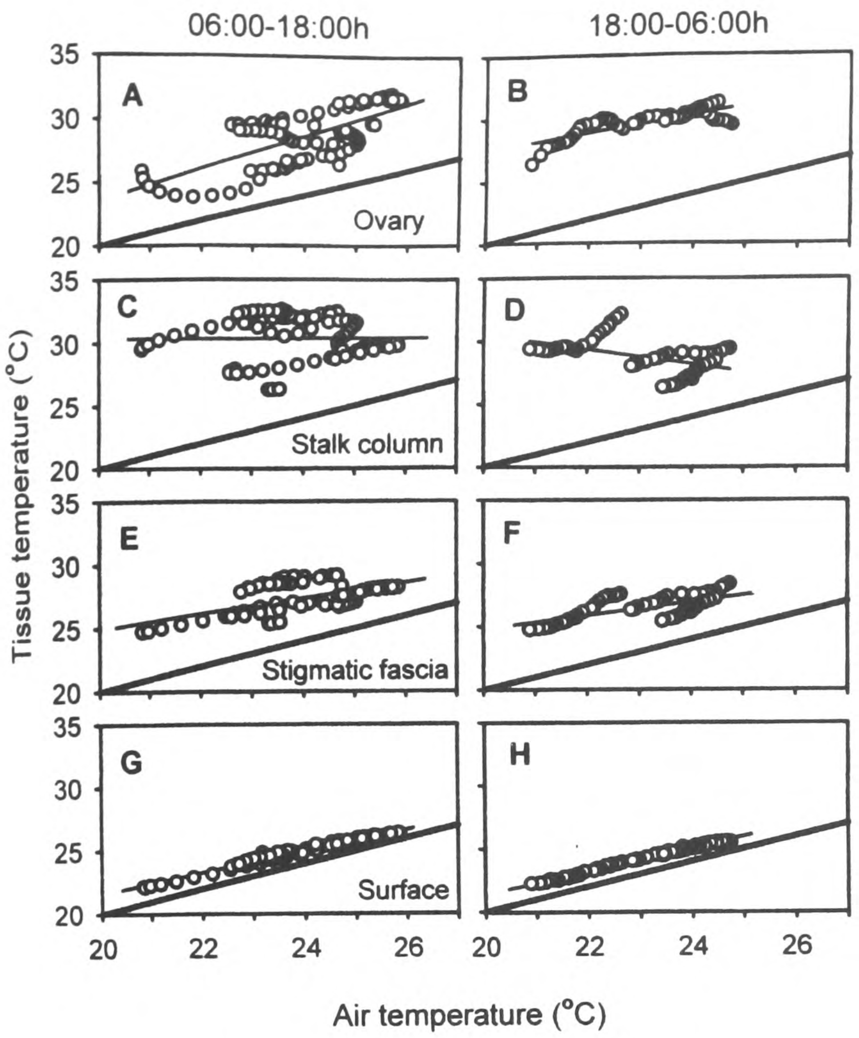

Fig. 4A–H Relationship between air temperature and tissue temperatures of a fresh female flower. To simplify the plots, data are plotted by five to seven readings of the original data. The *bold line* indicates the one-to-one relationship of air temperature. All internal parts were consistently warmer than the air and the stalk of the column shows evidence of thermoregulation. **A,C,E,G** Daytime. **B,D,F,H** Nighttime. **A,B** Ovary. **C,D** Stalk of the column. **E,F** Stigmatic fascia. **G,H** Surface

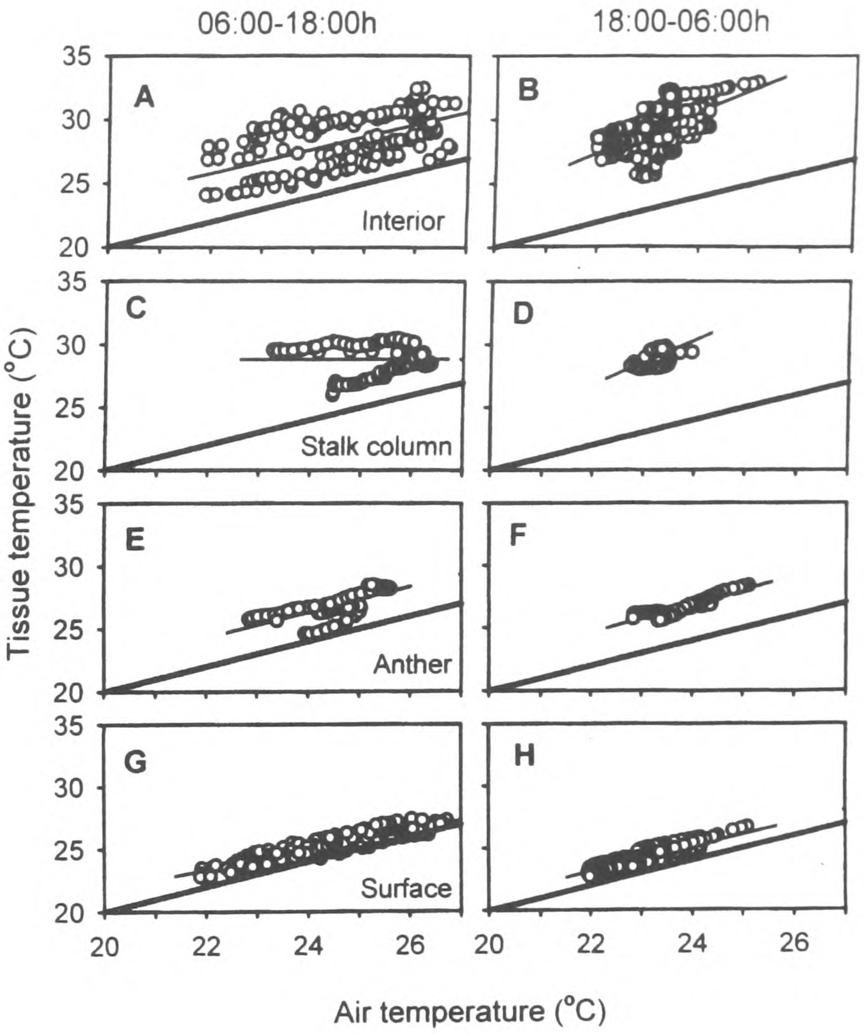

Fig. 5A–H Relationship between air temperature and tissue temperatures of a fresh male flower. To simplify the plots, data are plotted by five to seven readings of the original data. The *bold line* indicates the one-to-one relationship of air temperature. All internal parts were consistently warmer than the air and the stalk of the column shows evidence of thermoregulation. **A,C,E,G** Daytime. **B,D,F,H** Nighttime. **A,B** Interior. **C,D** Stalk of the column. **E,F** Anther. **G,H** Surface

To explore the relationship between tissue and air temperature, and to test the hypothesis of thermoregulation, we plotted the tissue and air temperatures as a linear regression for female (Fig. 4) and male (Fig. 5) flowers. The slope of the relationship was often very shallow, and in the case of the stalk of the column, tissue temperature hardly changed despite significant variation in air temperature. This indicates thermoregulation.

There was no obvious difference between the patterns shown in the male versus the female flowers (Figs. 4, 5). The highest temperatures were always found in the internal tissues whilst the surface temperature was closely coupled to air temperatures.

There was a lack of correlation between net radiation (measured with a Funk-type net radiometer) and excess temperature of bud and flower parts during the sequence of blooming (data not shown). This lack of correlation indicates that the high temperature detected in the flowers is endogenous, and not dependent on the radiation load.

Discussion

We have shown that *R. lowii* is an endothermic plant in which buds and blooming flowers constantly maintain their internal temperature several degrees above ambient. Endothermic activity was detected several weeks before anthesis, in young buds, progressively increased to reach its maximum 2 days before anthesis and persisted for some days after the flower had decayed.

One or 2 days before anthesis and during the 2 days after anthesis, the flower kept its internal temperature almost constant between 30 and 33°C when the air temperature fluctuated between 21 and 26°C. The relative constancy of internal flower temperature suggests thermoregulation by the flower. Many flowers have been demonstrated to increase their temperature above ambient as a consequence of endothermy, the heat being produced by cyanide-insensitive respiration (Meeuse and Raskin 1988; Skubatz et al. 1990). However, thermoregulation is unusual, implying the possession of a control mechanism. The support for thermoregulation in this study is that the flower maintains a relatively constant internal temperature regardless of the ambient temperature and the net radiation of the flower.

By comparing the 1:1 regression lines in Figs. 4 and 5, we conclude that for both female and male flowers, the most endothermic and thermoregulatory part was the stalk of the column. This is located in the centre of the circumambulator (Fig. 1C) which is the area where the flies enter and, "by coincidence", their dorsal regions touch the stigma and anthers in female and male flowers, respectively, and effect pollination. Consequently, endothermy and thermoregulation in *R. lowii* seem to be related to two phases of the pollination process. First, endothermy may be connected to the volatilisation of odorous chemicals that contribute to the long-distance attraction of the pollinators (unpublished data). On the other hand, it also seems to be connected to the respiratory release of CO_2 which, in combination with other volatile chemicals produced by the flower, may stimulate oviposition. Such a synergistic effect by CO_2 has been shown in *Lucilia cuprina* (Wiedemann) (Barton Brown 1979), although this species lays on live sheep which emanate CO_2 while the pollinators of *Rhizanthes* are carrion breeders. Oviposition may indirectly enhance pollination by keeping the flies searching longer for suitable sites to lay, one of the best being the circumambulator, entering and leaving of which is a precondition for pollination.

Endothermy was detected in young buds and decaying flowers as well as in mature flowers. To what extent is endothermy in the young buds an adaptation to survival? We suggest two possible alternatives. One is that the "machinery" for producing heat is formed early in life, and production of heat is then incidental because it is of no cost for the plant itself (being a parasite, it obtains its energy source from its host), although the host has a limited source of energy. Therefore, it may not have implications for survival.

The second alternative is that endothermy of the buds assists the release of defence volatiles. This seems less likely, as in a 1-year study at Kuala Belalong, bud mortality was as high as 90% and was attributed to fungal or bacterial attack (Awg Samhan Nyawa, personal observations). Moreover, during 1998, the few buds observed were eaten by bearded pig (*Sus barbatus*) (S. Patiño, personal observations).

Our results suggest that there are no significative differences in the patterns of endothermy and thermoregulation between female and male flowers. For both flower types, the ovary and interior became the most endothermic part after anthesis. The continuing endothermy after the flowers decayed in the case of the female flowers may help to promote the growth of the pollen tube and development of the seeds.

Detailed studies by Bänziger (1996) on the pollination ecology of *R. zippelii* revealed that this is a sapromyophilous flower in which pollination is based mainly on brood-site deception, and only to a small extent on food reward (sugars produced at the nectar pads). We observed the same pollination syndrome in *R. lowii*. It seems that the genus *Rhizanthes* does not offer heat as a reward (because the air temperatures in the forest are sufficient for normal flight). Moreover, the food reward appears to be of secondary importance to the pollinator flies.

There may be some common characteristics among endothermic plants (Seymour and Schultze-Motel 1997), but *Rhizanthes* seems not to comply. For example, most thermogenic flowers are monoecious and protogynous (female flowers mature before male flowers), while the flowers in this study were all unisexual. Endothermy has also been suggested to be present only in ancient families of flowering plants. Whether the Rafflesiaceae are among the most primitive angiosperms is unclear, as parasitism involves dramatic morphological and molecular changes (Nickrent 1996).Whether this family belongs in or near the Aristolochiales or is a member of the Rosidae remains a matter of debate (Cronquist 1981; Endress 1994; Thorne 1992). In any case, it seems that endothermy in flowers is a homoplastic character present in different taxonomic groups not necessarily congruent with a similar morphological organisation.

Acknowledgements S. Patiño was funded from Colombia by a Colciencias scholarship and support by the Instituto de Investigación de Recursos Biológicos "Alexander von Humboldt". We acknowledge financial support from the Davies Expedition Fund and Development Trust of the University of Edinburgh. At the Universiti Brunei Darussalam, thanks to Dr. Peter F. Becker for stimulating discussion and encouragement and Dr. Melvin T. Tyree for lending us his laboratory and equipment, Awg Samhan b Nyawa for providing access to his unpublished data, to the biology department staff for their help during this work, to the staff at Kuala Belalong Field Studies Centre, especially Dr. Colin Maycock and Ramlah for locating the sites where *R. lowii* grows and Dr. Maurizio Mencuccini for artistic support.

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