Available online at www.sciencedirect.com

ScienceDirect

South African Journal of Botany 76 (2010) 717–725

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

Scent chemistry and patterns of thermogenesis in male and female cones of the African cycad *Encephalartos natalensis* (Zamiaceae)

T.N. Suinyuy [a,d,c,*], J.S. Donaldson [a,b,d], S.D. Johnson [c]

[a] KRC, SA National Biodiversity Institute, Private Bag X7, Claremont 7735, Cape Town, South Africa
[b] Research Associate, Fairchild Tropical Botanic Garden, Miami, FL, USA
[c] School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3201, South Africa
[d] Department of Botany, University of Cape Town, South Africa

Received 20 June 2010; received in revised form 11 August 2010; accepted 19 August 2010

Abstract

Most, if not all, extant cycads are pollinated by insects which use the cones as larval brood sites. These interactions appear to be mediated by cone volatiles, and, in some species, by patterns of thermogenesis. We investigated the chemical composition of volatile emissions and patterns of thermogenesis in cones of the South African cycad *Encephalartos natalensis,* using a gas chromatograph–mass spectrometer (GC–MS) and miniature temperature data loggers (ibuttons), respectively. This was done during various developmental stages (before and during receptivity and pollen release) for both female and male cones. A total of 31 compounds were identified in headspace samples; 17 of which were common to both sexes, 12 found only in male cones, and two found only in female cones. The major volatiles in pollen and female cones are (3*E*)-1,3-octadiene (averaging 54.25% and 15.82% of total emissions), (3*E*,5*Z*)-1,3,5-octatriene (averaging 13.37% and 47.66%), and α-pinene (averaging 16.29% and 12.24%). Female cones were not thermogenic before and during receptivity whereas pollen cones were thermogenic during pollen shedding. Thermogenesis of male cones occurred between 1400 h and 1530 h on successive afternoons, reaching an average of c. 10.5 °C above ambient temperature. Volatile emissions and thermogenesis occurred in association with insect activity on the cones suggesting that they both play a role in regulating insect behaviour.

Keywords: African cycads; Gas chromatography–mass spectrometry; Insect visitors; KwaZulu-Natal; Pollination; Thermogenesis; Volatile emissions; Zamiaceae

1. Introduction

Several highly specialized plant–pollinator interactions have been shown to be mediated by emission of volatiles in conjunction with heat production (Bronstein et al., 2006; Jürgens, 2009; Pellmyr and Thien, 1986; Pellmyr, 1992; Seymour and Schultze-Motel, 1997). Although this area of research has focused mostly on flowering plants, there is an emerging body of work on cycads, which shows that volatile emissions and thermogenesis also influence pollinator behaviour in these early seed plants (Donaldson, 1997; Seymour et al., 2004; Tang, 1987a,b; Terry et al., 2004, 2007). Studies of cycads can provide important insights into the evolution of plant–pollinator interactions. Firstly, cycads are the oldest group of extant seed plants and the evolution of insect pollination mutualisms in cycads may have pre-dated the evolution of insect pollination in angiosperms (Norstog and Nicholls, 1997; Stevenson et al., 1998). Secondly, it has been hypothesized that plant volatiles originated as herbivore deterrents and later served a function as attractants (Pellmyr and Thien, 1986). All known cycad–pollinator interactions involve insect herbivores that feed on cycad reproductive structures, and this means that cycads are a good model to test this hypothesis. Finally, studies of cycad–insect associations indicate that some cycad–pollinator interactions could have originated through a shift from angiosperm host plants to cycads (Oberprieler, 2004). Therefore, studies of cycads may contribute to a better understanding of the evolution and function of volatile compounds in herbivore and pollinator attraction, and the occurrence of host shifts.

---

[*] Corresponding author. KRC, SA National Biodiversity Institute, Private Bag X7, Claremont 7735, Cape Town, South Africa.
E-mail address: T.Suinyuy@sanbi.org.za (T.N. Suinyuy).

0254-6299/$ - see front matter © 2010 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2010.08.014

Early observations of African cycads indicated that male cones emitted volatiles and produced heat (thermogenesis) during pollen shed, and that insects visited male and female cones at the time of odour emission and heat production (Jacot-Guillarmod, 1958; Pearson, 1906; Rattray, 1913; Tang, 1987a). However, the role of insects and the function of volatile emissions and thermogenesis in cycad pollination went untested for a long time because of the prevailing paradigm that all cycads were wind-pollinated (e.g. Chamberlain, 1935). Subsequent studies have shown that at least four African cycad species, *Encephalartos cycadifolius*, *E. villosus*, *E. friderici-guilielmi* and *Stangeria eriopus,* are pollinated by beetles (Donaldson et al., 1995; Donaldson, 1997; Proches and Johnson, 2009; Suinyuy et al., 2009). These studies, together with those of other extant species (e.g. *Zamia furfuracea*, Norstog et al., 1986; *Z. pumila*, Tang, 1987b; *Macrozamia communis*, Terry, 2001; *Bowenia*, Wilson, 2002; *Lepidozamia peroffskyana*, Hall et al., 2004; *M. machinii* and *M. lucida*, Terry et al., 2005; and *Cycas*, Kono and Tobe, 2007) provide experimental evidence for insect pollination in seven cycad genera and strong circumstantial evidence for insect pollination in the remaining three genera, *Ceratozamia* and *Dioon* (Vovides, 1991), and *Microcycas* (Vovides et al., 1997). Wind pollination, if it occurs, is likely to be an exception to this general pattern of insect pollination, or may occur in conjunction with insect pollination (Niklas and Norstog, 1984). Although insect pollination is apparently common in extant cycads, the role of cone volatiles and thermogenesis in mediating cycad–insect interactions is still poorly known.

Early studies of other African *Encephalartos* cycads have identified thermogenesis in male cones of *E. altensteinii* and *E. lehmannii* (Jacot-Guillarmod, 1958) and in male and/or female cones of *E. barteri*, *E. bubalinus*, *E. ferox*, *E. gratus*, *E. hildebrandtii*, *E. longifolius*, and *E. manikensis* (Tang, 1987a). Gas chromatography–mass spectrometry (GC–MS) analyses of volatiles have been conducted only for *E. altensteinii* and *E. villosus* (Pellmyr et al., 1991; Suinyuy et al., in press). Therefore, further studies are required to determine the possible role that cycad cone volatile and thermogenesis plays in regulating insect behaviour in *Encephalartos*.

In this study, we examined cone volatiles, and thermogenesis, as well as insect visitors, in *Encephalartos natalensis* Dyer and Verdoorn*,* a cycad in which both female and male cones are known to be visited by a variety of insect species (Oberprieler, 1995; Vorster, 1995). In our study, we measured thermogenesis, analysed volatile compounds, and surveyed insect visitors in both male and female cones at different stages of development (before and during pollen shedding and receptivity). We compare the results from these studies to the available data for other cycads and discuss the possible function of cone volatiles and thermogenesis for pollination.

2. Materials and methods

2.1. Study species and system

E. natalensis, commonly called the Natal cycad, is endemic to South Africa and is widely distributed in KwaZulu-Natal. The species occurs mostly inland and is often associated with rocky outcrops, cliffs and escarpments in hilly terrain (Jones, 1993). The climate in this area is characterised by hot and wet summers with cold and dry winters and occasional frost. Different forms exist that are differentiated mostly by leaf characteristics and the extent of woolly tomentum in the crown (Giddy, 1978; Goode, 2001). We sampled *E. natalensis* specimens of unknown origin at the Pietermaritzburg campus of the University of KwaZulu-Natal (UKZN), as well as plants occurring naturally at the KwaZulu-Natal National Botanical Garden, Pietermaritzburg, and the conservation area of Hilton College just north of Pietermaritzburg. Mature specimens have an erect or reclining stem and older plants can be between 4 and 6 m tall (Fig. 1a). Female and male plants produce between one and five cones per stem. Female cones are 50 to 60 cm long and 25 to 30 cm in diameter while male cones are 45 to 40 cm long and 10 to 12 cm in diameter (Fig. 1a,b). Female and male cones have a woolly tomentum early in their development but gradually lose their woolliness as they mature (Giddy, 1978). Male cones are pale yellow when mature and the cone extends so that the sporophylls separate and pollen is freely dispersed from the cone (Tang, 1987a).

2.2. Sampling of volatile compounds

Headspace sampling was used to collect volatiles from male and female cones before and during pollen release and receptivity, respectively. Polyacetate bags (Nalo Bratfolie Kalle GmbH-Germany) were placed over the cone just prior to sampling in

Fig. 1. (a) Female *Encephalartos natalensis* with receptive cones; (b) male *Encephalartos natalensis* with male cones at pollen shed stage.